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Relations of hormones to correlation in maize

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RELATIONS OF HORMONES TO CORRELATION IN MAIZE

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INTRODUCTION

Growth correlation in plants is defined as interaction in the development of various parts and organs, or as the effect of one plant part upon the development of another. Hormones are classed as correlation carriers, although their specific action is in most cases unknown.

Seedling maize plants show a well developed correlation between the development of the coleoptile and first internode, and of the other organs of the plumular axis. Coleoptile growth is prominent in the early stages of germination with a gradual shift to rapid intercalary growth in the upper portion of the first internode at a point just below the coleoptilar node. During these phases growth of the plumule and the nodal roots is largely inhibited. Under field conditions the emergence of the coleoptile tip marks the sudden, irreversible end of internode growth and the beginning of rapid growth of the plumule and nodal roots.

Plant hormone literature is concerned very extensively with the production or activation of growth hormone in the coleoptile tips of the Gramineae. Van Overbeek (52) and Inge and Loomis (24) have suggested that changes in the hormone output of the coleoptile tip are responsible for the shift from internode to plumule growth at the time of emergence, and have shown that illumination or other treatments of the tip intended to reduce its hormone production will bring about the growth shift. Inge

and Loomis, however, made no hormone tests to substantiate their theory and Goodwin (22) has more recently reported indications of the action of factors other than diffusible auxins.

The present research was undertaken to clarify the relationships involved, and particularly to study changes in the hormone production of maize coleoptile tips under illumination and their correlation with various growth responses. We have been interested also in changes in hormone production with senility, since the shift to plumule development is eventually made, even in complete darkness, under conditions which suggest that the aging coleoptiles are no longer effective inhibitors of plumule growth.

REVIEW OF PERTINENT LITERATURE

Much of the literature regarding growth hormones in plants has been adequately reviewed by Went and Thimann (62 - 560 references), and Boysen Jensen (15). Our discussion will, therefore, be confined to that literature which is more directly concerned with the problem of the relation of plant hormones to growth correlation in maize.

Duhamel Du Monceau (62) and Sachs (62) were among the early investigators concerned with correlation in plants. Du Monceau thought of correlation as brought about by two saps, one moving downward, the other upward. The downward moving material was considered to be elaborated in the leaves and passed through the phloem for the nutrition of roots. Sachs assumed the existence of organ-forming substances, which, in minute amounts, directed development. He assumed, also, the polar distribution of these substances, and that their distribution could be modified by external forces, such as light and gravity.

Murneek (33) was concerned with the general phenomenon of correlation, and studied the mechanism through which these growth reactions found expression in the plant. His investigation (34) on the effects of correlation between vegetative and reproductive functions in the tomato formed a background and furnished an impetus for future investigation of various types of correlation. His results support the concept of correlation as competition for food materials. He found that a maximum crop of fruit had a strikingly retarding effect on vegetative growth and development. The fruit diverted and monopolized in some manner all of the available nitrogen,

accounting, in part at least, for marked carbohydrate accumulation in vegetative structures. Although Murneek included other possibilities, his suggestion of a controlling glandular organization or system of secretions operating in this correlation was an idea which was relatively new at the time.

Dostal (59) showed that growth rates of different organs of a young seedling could be changed by application of auxin to its different parts; this was an indication that the correlation between organs of the seedling was perhaps also normally controlled by auxin. There is not, however, a considerable literature on the relation of plant growth hormones to correlation in seedlings.

Inge and Loomis (24) after a study of the distribution of growth of maize seedlings between plumule, mesocotyl, or first internode of the epicotyl, and the roots, presented an hypothesis based on the relation of growth hormones to these correlative developments. These investigators found that elongation of the first internode of the epicotyl of germinating maize was stopped by exposing the coleoptile tips to light, decapitating the coleoptile, or heating emerging coleoptiles at 50 degrees for one hour. However, application of heteroauxin paste to the tips at the time of illumination prevented the inhibiting effect of light. During the normal period of rapid internode elongation, plumule and nodal root development was inhibited, but checking the internode growth by illumination stimulated plumule and root growth, and after 24 hours the shift became irreversible. The explanation presented was as follows: internode elongation is dependent on a constant supply of auxin from the coleoptile tip. This same auxin

supply inhibits plumule and nodal root growth. When the supply of auxin is reduced temporarily, plumule development begins and the first internode of the epicotyl loses its capacity for further elongation.

It appears that the capacity of auxin to act as a correlation carrier in the seedling is conditioned by various factors, of which light seems of primary importance. The light studies made on grass seedlings, then, may be considered in terms of hormones and growth. Most of these concern the oat seedling, but the similarity in structure of oats and corn is sufficient to warrant their inclusion.

The general anatomy of the maize plant has been reviewed by Toole (50), Martin and Hershey (32), and Boyd and Avery (14). Avery (3) thought there was sufficient evidence to consider the coleoptile a single leaf rather than the result of fusion of two stipules or other structures. Then, in a comparative anatomy of the embryos and seedlings of grasses (4), anatomical relationships were further clarified. The scutellum in maize, oats, and wheat is the cotyledon. The coleoptile is homologous with a foliage leaf and is the second leaf of the plant. In the maize and oat seedling the elongated structure between the cotyledon and the coleoptile is the first internode of the axis. The term "mesocotyl" has been applied to this structure, although this name is now meaningless. The first internode has the function of raising the coleoptile with the embryonic shoot through the soil nearly to the surface, where it may develop normally. Perry (37) claimed evidence for a possible hormone secreting region in the grass coleoptile. He concluded that an adequate histological basis existed for the secretion and transportation of a growth hormone in the coleoptile of the plants studied.

Tetley and Priestley (44) showed that light facilitated elongation and made it proceed more rapidly from apex downward by increasing tissue permeability. However, it diminished the final length of the coleoptile by decreasing the time over which elongation went on. Avery and Burkholder (7) showed that cell division takes place in the early stages of coleoptile growth, but that this cell division ceases relatively early, so that by the time the coleoptile is used for auxin tests its elongation is directly proportional to the increase in length of its constituent cells. Most of the increase in number of cells takes place by the time the coleoptile is 1 cm. long. Further slight increase in numbers of cells takes place before the coleoptile is 18 mm. long.

Although Rothert (62) was the first to recognize that one effect of light on growth of the internode is to check the elongation, further investigations did not follow until a much later date. Some investigators studied both the coleoptile and the internode; others either of the two. The results obtained are usable in interpreting correlative phenomena in the seedling.

Certain investigators were primarily concerned with the effect of different wave lengths on the growth of coleoptile and mesocotyl. Du Buy (19) exposed plants of Avena sativa to heat rays and showed that light from which the heat rays were absorbed was able to inhibit the growth of the mesocotyl; red was most effective, blue least. The research of Avery, Burkholder, and Creighton (8) is in accord. They found that in darkness the first internode grew to a greater length than the coleoptile, whereas under light of all intensities and qualities studied the coleoptile grew to a greater length than the first internode. Growth of the first internode

was reduced appreciably by low intensities of all wave lengths; at equal intensity, red was most effective in inhibiting elongation, green and blue were next with violet following. High-intensity light inhibited growth of the first internode more than the coleoptile. Cell size and number were correlated with length of the first internode.

Johnston (25) studied the growth of the Avena coleoptile and first internode in different wave length bands of the visible spectrum. As compared with darkness, radiation of low intensity greatly depressed the growth of the first internode, and the ratios of first internode and coleoptile to total length were extremely critical indices of intensity of illumination. Apparently growth of the coleoptile retarded growth of the first internode,and growth of the one tissue took place at the expense of the other. Johnston was of the opinion that light probably acted more as a redistributing agent of the growth substance than as an inactivating agent. However, even at the low intensities employed, there was a slight indication that some inactivation occurred, since the total average length of the illuminated seedlings was slightly less than that of the dark controls.

Avery and his co-workers (9) made considerable contributions to the effect of light on the mesocotyl and coleoptile, with Avena as the test object. Final length of the first internode grown under a series of different light intensities varied inversely with the intensity. Early stages of germination in Victory oats in light (1000 wat Mazda) and in darkness showed that the internode elongated slightly during early swelling. In darkness further polarized growth occurred in the internode, but in strong light growth ceased early and shifted to the coleoptile. Different

amounts of light influenced polarized growth in different organs and tissues in different ways. Very low intensities of light inhibited growth of the first internode, but not that of the coleoptile; high intensities inhibited the internode and appreciably shortened the coleoptile. In the complete absence of light the internode grew extensively and the coleoptile was somewhat shorter than in plants which received small amounts of light in the early stages of germination.

The effect of light in shortening the first internode of the axis was brought about primarily by a reduction of cell division. It was suggested that the influencing factors are probably concerned with certain substances necessary for cell division, and if so, such substances are rendered ineffective, or are changed in their path of movement, by very low intensities of light.

Araki (1) made physiological-anatomical investigations on the growth of the mesocotyl in darkness and light, and divided the mesocotyl into three types on this basis. Araki and Hamada (2) studied localization of the light sensitive zone of seedling organs of Avena sativa. They illuminated coleoptile tissue and noted the resulting inhibition of the mesocotyl.

By study of isolated sections of both mesocotyl and coleoptile Schneider (40) showed that each organ could act as its own receptor of the light stimulus. Thus there was a direct mechanism for inhibition of growth of the mesocotyl and for the acceleration of the growth of the coleoptile during early stages of elongation. Although growth of the mesocotyl tissue was shown to be a function of the concentration of auxin supplied, no support was found for the existence of an indirect mechanism involving

a receptor in some remote part of the seedling and mediated by way of auxin. This view appeared in direct opposition to that of Inge and Loomis (24).

Other studies were more directly concerned with the internode alone. Schneider (39) investigated light as a direct, controlling factor in the growth of the first internode of Avena. He found that growth of the first internode of some of the Gramineae was highly sensitive to light, elongation being almost completely inhibited by exposure, even to dim red light. Straight internodes with a minimal inhibition of growth were obtained by giving a one- to two-minute exposure to very dim red light at about thirty hours after germination. When the internodes had elongated considerably, sections three millimeters in length were cut from them and allowed to grow in the presence of appropriate concentrations of auxin. The elongation of such sections was markedly inhibited by red light even in the presence of auxin. Growth inhibition was largely a direct effect on the tissue of the internode itself.

Goodwin (22) distinguished two phases in the inhibition of the first internode of Avena by light. The first phase, high sensitivity to radiant energy, was due to the suppression of cell division. Wave lengths producing this inhibition ranged from the ultra-violet to the long infra-red, but infra-red radiation was very much less effective. The extent to which cell division was inhibited by red light depended directly upon the amount of radiant energy received by the plant. The second phase, much lower sensitivity to radiant energy, was due to a reduction of cell elongation. Wave lengths longer than 16,000 Å had practically no effect on cell elongation at the intensities used.

Goodwin reported that the radiant energy causing inhibition of the first internode may be received by the plant either below or above the coleoptilar node. His conclusions were more in keeping with Inge and Loomis than with Schneider. When the coleoptile tip alone was irradiated the inhibition was apparently indirect, the radiant energy bringing about some chemical change in the coleoptile tip which was subsequently transmitted to the internode.

Other work was more concerned with the coleoptile. Lange (28) studied the distribution of light-sensitivity in the tip of the oat coleoptile. Van Overbeek (53) studied light-growth response and auxin curvatures of Avena. Burkholder and Johnson (16), in research on the inactivation of plant growth substance by light, showed that light destroyed the activity of growth substance in the coleoptile tips of Avena and Zea.

Oppenworth (36) made an interesting contribution to the subject of photoinactivation of auxin in the coleoptile of Avena. Avena coleoptiles were unilaterally illuminated with light from a mercury bulb. Immediately after illumination equal amounts of auxin diffused out of the illuminated and shaded sides. One hour after illumination only 56 per cent of the auxin of the unexposed controls was obtained from the illuminated side whereas 131 per cent was obtained from the shaded side.

Katunskij (27) illuminated coleoptiles of oats with electric vapor lamps. Illumination of short duration caused an almost instantaneous fall in the content of growth substance, while further illumination had little effect. He concluded that the formative effect of short illumination-elongation of the stalks--development of the leaf surface area, and differentiation of the conducting tissues -- depends directly on the decrease

in the content of growth-promoting substance caused by it. Upon anatomical examination those variants which had shown greatest growth, were, at the same time, characterized by the least differentiated conducting system. Etiolated plants differed from those cultivated under normal conditions of illumination in their higher content (overproduction) of growth substance under conditions of darkness or insufficient illumination. Since continued illumination was not necessary to keep the content of growth-substance in etiolated seedlings at a low level, short periodical illumination may be used as a means of controlling the undesirable phenomena of etiolation.

Naves (35) showed by the Avena test that apical portions of seedlings of Lupinus albus, grown under constant conditions in an illuminated thermostat, released to agar blocks a larger amount of auxin than dark-grown seedlings. This information linked auxin formation directly with photosynthesis.

The investigations of Skoog (41) showed that auxin is inactivated in solutions by modified dosages of X-irradiation. Extracted and synthetic heteroauxin, beta-indoleacetic acid, as well as auxin a were similarly inactivated by action of X-rays in water solution. In aqueous solution immediate inactivation occurred only in the presence of oxygen; a comparable inactivation was obtained in white light by the use of small concentrations of eosin as a catalyst. The growth hormone normally present in the plant was partially inactivated by modified dosages of irradiation, but in the Avena coleoptile there was no effect of irradiation on the formation of auxin by the plant. In green plants grown in the light, the formation of auxin was inhibited by the modified dosages used. The mechanism of transport

of auxin in the plant was not affected by irradiation. However, where irradiation reduced the amount of auxin, the mechanism of the effect was complex, involving the destruction and the inhibition of the formation of the growth hormone with a corresponding inhibition of the elongation of the internode, as well as markedly reduced formation of new tissues in the apex of the plant.

Van Overbeek (51) found hormones and the dwarf type of growth in corn intimately related. Length of the coleoptile is the same in dwarf and normal corn, but there is a difference in the mesocotyl. A smaller amount of auxin is available for the dwarf mesocotyl with resulting smaller growth. The reduced amount of growth substance can be explained by a higher destruction of growth substance in the dwarf. In another paper (55) Van Overbeek further corroborated that seedlings of this dwarf variety produced less growth hormone than normals, also responded less to applied hormones, and their tissue was unusually active in the destruction of hormones.

Van Overbeek (52) exposed young maize seedling to 48° C. in air for about one half hour and showed that mesocotyl growth was inhibited. The amount of growth substance given off by the coleoptile tips was less in the heat treated plants than in controls, and by applying extra growth substance to the tips of heat treated plants it was proven that inhibition of mesocotyl growth was due to the decreased amount of growth hormone given off by the tip. Thus he favored an indirect mechanism for inhibition of the mesocotyl.

Thimann and Schneider (48) noted that the entrance of indole-3-acetic acid into plant tissue took place at least as readily through cut and wounded surfaces as through intact epidermis; the entry was more rapid

through wounded surfaces, but the final state reached was probably the same in each case. An explanation of the cause of the tissue tensions of older botanists was suggested by the fact that inner and outer layers of tissue behaved differently in auxin; the inner layers showing most of their auxin response in very low concentrations, the outer layers in very high concentration.

Du Buy (20) found that the cells of the coleoptile became less sensitive to applied auxin as they were farther from the auxin-producing zone. In plants of the same age, physiological aging was produced by decapitating and then preventing the auxin from regenerating by decapitating a second or third time after suitable intervals. This treatment increased the response to applied auxin because the auxin content became very low, but if lack of auxin continued the plants soon became less sensitive or "aged."

Further, Du Buy (20) stated that this age effect is due to an irreversible change in the elasticity and extensibility of the cell wall. He showed that with increasing age the response of Avena coleoptiles to auxin decreased for all concentrations of this growth regulator, and that the production of growth regulators decreased with increasing age. The auxin concentration which just causes a visible curvature ("threshold concentration") varies with the conditions of the reacting cells and increases with the age of the plant; it also varies with the properties of the organ -- thickness, speed of transport, etc. It is only in a certain stage of their development that plants are sensitive to auxin; if these substances are applied before or after this period of sensitivity, the response decreases or disappears totally.

Went (61), by artificially increasing the auxin concentration in a coleoptile, showed that in normal growth auxin is limiting for growth of practically all cells, either directly as a growth promoter or indirectly as an inhibitor of aging. He considered that aging is due to cell wall formation exceeding the rate of elongation. In the reverse phenomenon, rejuvenation, there was a gradual increase in the growth rate of aged cells after an excess of auxin had been applied. Cells which had stopped growing under the combined effects of aging and auxin deficiency resumed growth with an excess auxin supply.

The phenomena of aging entered also into the work of Bonner (12). He placed segments taken from the tips and bases of coleoptiles in solutions of growth hormone. Segments from the upper one third of the coleoptile showed a growth response more than twice as great as that of the lower segments. Bonner offered the explanation that mature tissues near the base are incapable of growth when the food supply is exhausted, even in the presence of favorable concentrations of hormone. Went discussed this food factor as a factor limiting growth along with age and auxin. Food is stored in the seed and increases both leaf and coleoptile growth. Under normal conditions the food factor is in relative excess in the seedling but becomes limiting upon application of excess auxin or after deseeding.

Van Overbeek (55) was able to show that production of auxin varies with the age of the seedlings. Maximum production was observed on the fifth to the sixth day after sowing.

In the general phenomenon of correlation we think of inhibition of

growth as related to acceleration of growth. Loeb (62) studied correlative inhibition in Bryophyllum. He noted that if one shoot of the plant was growing rapidly, it deflected food substances away from other buds which thus were inhibited. Later Loeb attributed bud inhibition to the influence of special inhibiting substances formed in the leaf and transported basipetally in the stem. Loeb's study of buds suggested that the apical buds send out substances toward the base of the stem which prevent other buds from growing.

Uhrva (62) found that a substance diffusible into agar or gelatin was present in the leaves of Bryophyllum, and that this substance inhibited lateral bud development, thus supporting the work of Loeb. Uhrva also reported that the hormone from Avena coleoptile, diastase, and saliva, as well as acids, had the same effect. Snow (43) also presented evidence of special inhibiting substances.

The research of Thimann (46, 47), and Thimann and Skoog (49) was primarily concerned with the inhibition of the development of lateral buds and shoots by growth hormones. These investigations throw some light on the mechanism of inhibition as it is exemplified in some experiments in this paper. However, Snow (42, 43) and Le Fanu (29) seem to have been the main workers interested in inhibition in coleoptiles and stems.

Snow (42) applied a paste of heteroauxin in lanolin near the bases of decapitated, dark-grown oat coleoptiles. He found that heteroauxin could be transported to some extent in the morphologically upward direction in coleoptiles and that this upward transport took place largely in the conducting strands. In whichever direction the heteroauxin was transported in coleoptiles it accelerated their growth. Then, using pea seedlings,

this investigator found that the retardation of very young internodes by heteroauxin drawn up with the transpiration stream is probably brought about in a different way from retardation of internodes by heteroauxin paste.

Le Fanu (29) concluded from her investigation that the nature of auxin action is determined by the position of the auxin source relative to the organ affected. She found that heteroauxin on stems below the elongating region retarded elongation of the parts above, although the same paste applied from above accelerated elongation. Above the auxin paste the preliminary acceleration was due to a little of the heteroauxin traveling upward; the retardation was an indirect effect similar to correlative inhibition. But when the paste was applied above the elongating zone, heteroauxin traveling down through this zone in higher concentrations prevented the inhibiting influence from producing its effect. The possibilities were presented, however, that either auxin itself travels upward and has a different effect from the same substance traveling downwards, or the auxin itself traveling only downwards produces a secondary inhibiting effect which can travel in either direction; yet there is no evidence of a special substance capable of inhibiting and produced secondarily by auxin action. Experiments showed very little auxin in any inhibited shoots, but it was difficult to show that there was none.

Bonner and Koepfli (13) present evidence for the inhibition of root growth by auxin. Within suitable concentration ranges auxins promote and are essential for the growth of many stems, petioles, and coleoptiles of grasses, etc. On the other hand, in similar concentrations they inhibit the growth of roots. Thus auxin enters into an initial reaction which is common to root inhibition and shoot elongation.

Thimann (46) wrote a general paper on the nature of inhibitions caused by auxin. In view of the parallel behavior of roots and buds in regard to auxin inhibition, and the fact that very dilute auxin solutions increased root elongation, it was suggested that roots, buds, and stems all behave in a comparable way, their growth being inhibited by relatively high and promoted by relatively low auxin concentration. The differences between them are of a quantitative rather than a qualitative nature. On this basis, the responses of different organs to auxin are presented as a series of optimum curves of similar shape.

Although the only roots concerned in this study are the nodal roots, it might be well to mention some of the literature on auxins and root growth. Went (63) states that auxin is only one of the many specific internal factors involved in root formation. In addition to the chemically well-defined factors, such as auxin, biotin, carotin, folliculin, vitamin B1, and amino acids, certain experiments have pointed toward the existence of a special root forming hormone, rhizocaline, a special substance considered to cause the initiation of roots. The experiments suggested that the rhizocaline is redistributed inside the plant under the influence of auxin.

Van Overbeek (54) studied the effect of roots on the production of auxin by the coleoptile. Removal of the root system for a period of 15-20 hours reduced the auxin production of the coleoptile tip of Avena seedlings markedly. This reduction caused a decreased growth, but an increased sensitivity to auxin.

Later (56) Van Overbeek raised the question of whether auxin is produced in roots. Although under the conditions described in his experiment

no evidence was found for auxin production, he stated that this did not necessarily mean that under natural conditions (intact roots) no production would take place. He did state that he had obtained some evidence that excised pea roots grown in vitro under sterile conditions and in complete medium were able to synthesize auxin.

The phenomenon of correlation is very complex, involving the interaction of many factors, and, in fact, all of the life processes of the plant. Other references bearing on correlative development of maize will be mentioned in the discussion.

MATERIALS AND METHODS

Various methods have been used to test growth hormones present in plants. These consist chiefly of diffusion and extraction methods. According to Van Overbeek (59) auxin exists in the plant in three forms: bound or inactive, an active auxin complex, and free auxin in equilibrium with the first two forms.

Extraction methods make possible an assay of the total quantity of hormone in the tissue or organ being tested (5). Haagen-Smith, Leech, and Bergen (23) have summarized methods of obtaining auxin from plant material by diffusion, solvent extraction, in vitro chemical or enzymatic treatment followed by solvent extraction, or biological digestion. Certain single-solvent methods have been used; these have been evaluated by Avery, Creighton, and Shaluoha (10). Most consistent results were obtained when alcohol was used as a solvent. Water extractions yielded much more growth hormone, but results were not as consistent. In a later paper (6) the multisolvent method was evaluated. This consists of successive extractions of the same tissue with several different solvents, frequently alcohol, chloroform, and water; it is considered most effective.

The diffusion method enables one to measure the amount of free hormone that will diffuse from tissue into agar, and it indicates the relative concentration of hormone in the tissue (5). The diffusion method was used for these tests. All experiments were performed in the photographic darkroom at an average temperature of 25° C.

Except during the actual course of experimental illumination, plants were exposed to no light but the phototropically inactive red light and this was only used when manipulations were necessary in the darkroom. There were several advantages derived from the use of this red light. First, the Avena coleoptile is very sensitive to light of the shorter wave lengths. This, if it falls on the plant from one side, causes phototropic bending, while if symmetrically distributed it causes a decrease of sensitivity to the applied auxin. Red filters cut out the phototropically active wave lengths of incandescent bulbs, both from the Avena coleoptiles and corn seedlings. Then, too, the red light can be used to suppress the growth of the first internode of germinating oat plants, as substantiated by the work of Lange (28) and Du Buy and Nuernbergk (21). Crooked test plants are apt to result from an elongated mesocotyl. Oat test plants were therefore grown in red light, but maize seedlings were held in a dark chamber.

Variability of Avena hormone tests was studied at Utrecht by Kogl and his co-workers (62). These workers found that if the tests were performed at the same time of day the variation in sensitivity was small. The mean curvature for a given concentration of auxin varied from day to day and even from hour to hour. Van Overbeek (58) found minimum auxin production at twelve noon. Therefore, a uniform procedure was followed in these experiments and growth substance determinations were made at the same time every day in so far as possible, largely in the late afternoon or evening.

The experimental corn, a double cross (J 205 x WF 9) x (M 14 x CC 38), was obtained from Dr. G. F. Sprague. For all tests the corn was presoaked

for one hour at a temperature of 40° C. to encourage uniform germination. Corn for the carbon arc experiments was germinated on moist blotters in germination trays and irradiated with Superten Eveready carbons. For the other light experiments corn which had been planted one half inch deep in sterilized sand was illuminated with a two hundred watt Mazda bulb at a distance which gave a measured intensity of 100 foot candles. Further details relative to the treatment of the corn are given in the individual procedures presented in the section on experimental results.

Markton variety of oats was chosen for the hormone tests from a list of United States grown oats suitable for the test, prepared by J. Van Overbeek (57). Markton CI - 2053 of the United States Department of Agriculture, grown at Aberdeen, Idaho, was considered a variety with outstanding qualities for the Avena test. It was characterized by good shelling and leaf pulling qualities and very straight plants. Seed was obtained through the courtesy of Dr. H. C. Murphy.

The method of testing the hormones in these experiments was a modification of Went's standard Avena method (62), and the instruments used were of his invention. Went was first to obtain auxin from coleoptile tips of Avena; he found that auxin would diffuse into agar blocks, and that the blocks placed on coleoptile stumps caused a curvature proportional, within limits, to the relative concentration of growth substance in the block.

In preparation for the tests the necessary amount of pure Difco agar was freed of growth substances by washing in running tap water for several days, after which a sufficient volume of water was added to make it to

1.3 per cent. Then the melted agar was poured into cotton plugged test tubes and sterilized. It was remelted as needed for tests and poured into standard molds to harden. Protection from desiccation was afforded by glass germination dishes tightly covered with glass plates. All instruments used in the manipulations were thoroughly cleansed before each test.

Technique in hormone determination was practiced by running a series of experiments testing the effect of certain dilutions of indoleacetic acid. The general procedure outlined followed a period of preliminary experimentation.

The Markton oats were first dehulled. After they were soaked one half hour in tap water in daylight, they were placed grooved-side downward on moist filter paper in glass germinating dishes, or moist chambers, and exposed to photographic red light in the darkroom. When the seedling roots were a few millimeters long the plants were placed in glass holders, which had been previously dipped in paraffin in order to hold the seeds more securely and prevent water from creeping into the guide. The glass holders were supported by brass clips set in notches in wooden racks. The roots of the oats dipped into water in zinc trays. Oat seedlings were ready for use when averaging thirty millimeters in height. This height was considered optimum because plants were then far enough above the guide to make operation easy, and were at the same time high in sensitivity.

Straight and uniform plants were selected. By placing the brass clips at the proper angle, these test objects were carefully adjusted vertically in the holders several hours before decapitation, in order to eliminate the possible effect of any disturbance just before experimental operations were begun. Two decapitations were made at two hour intervals

with special decapitation scissors. Crooked plants were rejected at the time of the second decapitation. If there was any water of guttation on the plants before the operation, it was carefully blotted off with filter paper. The first decapitation removed one to two millimeters of the tip, the most active auxin producing zone (58). At the second decapitation approximately the next five millimeters were removed. A second decapitation two hours after the first was proposed by Dolk (17) in consideration of the regeneration of the production of growth hormone in the uppermost zone of the stump.

Following the experimental period of light or darkness, the tips from corn coleoptiles of desired age were removed and placed immediately on the agar plates in glass moist chambers; twelve tips were placed on each plate. This was done at the time the oats were decapitated for the first time. The germinating dishes were placed in the darkroom and diffusion from the corn coleoptiles was allowed to proceed in complete darkness. By the time the oats had been decapitated for the second time the corn tips had been on the agar for two hours, the time considered sufficient for maximum diffusion.

After the second decapitation the primary leaf was partially pulled from the oat coleoptile with the aid of cork-tipped forceps, and used as a support for the millimeter blocks of agar. At the end of the diffusion period the tips of corn coleoptiles were removed from the agar plates. With the aid of a cutting frame and a razor blade the agar was cut into twelve blocks of equal size. These were placed on one side of the decapitated coleoptiles.

Two hours after application of the agar blocks the resulting oat

coleoptile curvatures were measured. A transparent celluloid protractor was placed over the coleoptile. The angle of curvature was measured directly by matching the thin line on the celluloid arm with the axis of the curved organ. Negative curvatures were measured and averaged for each treatment. Various units have been proposed to denote the amount of growth substance present (15). The results of these experiments, however, are stated simply in terms of total average degrees curvature.

The typical curvature produced by auxin is a negative curvature, or away from the side with the agar block; the active substance causes an increase in growth on the side to which it is applied. Positive curvatures, toward the agar block, were occasionally observed but were not considered in these experiments. According to Seubert (62) positive curvatures are caused by growth-retarding substances. Thimann (45) attributed the absence of growth-promoting activity to the destruction of auxin by enzymes. Gorter (62) stated that whenever an extract caused positive curvature it did so within two and one half hours after application of the agar; the onset of regeneration taking place about two and one half hours after decapitation (17), or (30) regeneration being greater on the side without the block.

Occasional plants which did not curve, following the procedure of Went, were not included in the means. Results were made comparable by the fact that measurements of angles were made at the same time after application of the agar blocks.

When auxin paste was used it consisted of a 1:400 mixture of beta-indoleacetic acid in lanolin. When not in experimental use this was kept in the refrigerator in order that its activity might not be reduced.

EXPERIMENTAL RESULTS

The Effect of Illumination on Mesocotyl Growth

Experiments with a Mazda lamp. The corn for these experiments was planted about one half inch deep in four inch pots of sterilized sand, fourteen plants in a pot. Growth took place in germinating chambers in the photographic darkroom. When the seedlings averaged about 6 cm., eight pots were selected and divided into two sets. One set of four was used for growth studies alone; the other set of four was used for study of both hormones and growth. Within each set one pot was held as a control, and the others were illuminated one, six, or twenty-four hours.

The same schedule was followed from day to day with a total of nine groups of plants. Seedlings, to be used as controls, either for growth or hormone studies, were labelled in the photographic darkroom, and were never exposed to any light but the minimum of phototropically inactive red light necessary for manipulations. All of the plants to be illuminated were placed under the lamp at the same time, and two pots of corn were removed at the end of each experimental period; namely, one, six, and twenty-four hours.

For example, after one hour had elapsed, two pots of corn were removed from the source of illumination. One pot, containing undecapitated plants, was placed at once in the darkroom for growth studies alone. Since the corn in the other pot was to be used for hormone as well as growth studies, the tips of the coleoptiles were removed and placed immediately upon agar

blocks to be tested for hormones according to the standard procedure outline in the section on materials and methods. The decapitated plants thus obtained were then placed in the darkroom for comparison with the undecapitated plants. The same procedure was followed at the end of six hours and twenty-four hours for plants illuminated for these periods.

Two growth measurements were made on all plants. The first, which preceded the illumination, involved only the axis and internode, since the plumule had not emerged. The next growth measurement was made on the axis, internode, and coleoptile three days after illumination; the exposed plumule then included what was left after internode and coleoptile measurements together were subtracted from the axis.

Results are shown in Table 1 and Figures 1 and 2.

These data show that with Mazda illumination of the coleoptile for one hour, six hours, and twenty-four hours there was a progressive decrease in the final length of the internode. This effect was highly significant in plants illuminated six and twenty-four hours and compared with controls.

Growth measurements of intact plants were considered more valid than those from decapitated plants whose mechanism probably was disturbed by the operation. This fact was taken into consideration in interpreting the results of plumule growth in decapitated and undecapitated plants. There was a progressive increase in plumule growth with Mazda illumination of undecapitated plants. This increase was significant when plants illuminated six hours and twenty-four hours were compared with controls. Plumule growth differences were also significant when plants illuminated one hour

Table 1. Effects of Mazda Illumination on Growth of Maize Seedlings -- Average of Nine Experiments

	Undecapitated			Decapitated			
	Axis	Internode	Plumule	Axis	Internode	Plumule	Degrees curvature-hormone test
	Length in mm.			Length in mm.			
Control	155.2	55.0	100.2	159.9	41.5	118.4	20.5
One hour	157.9	42.2	115.7	175.4	31.9	143.5	12.0
Six hours	149.2	28.5**	120.7*	139.0	22.8**	116.0	8.5*
Twenty-four hours	161.1	9.9**	151.2*	151.5	9.6**	141.9	6.7*

*Variation from control significant.

**Variation highly significant.

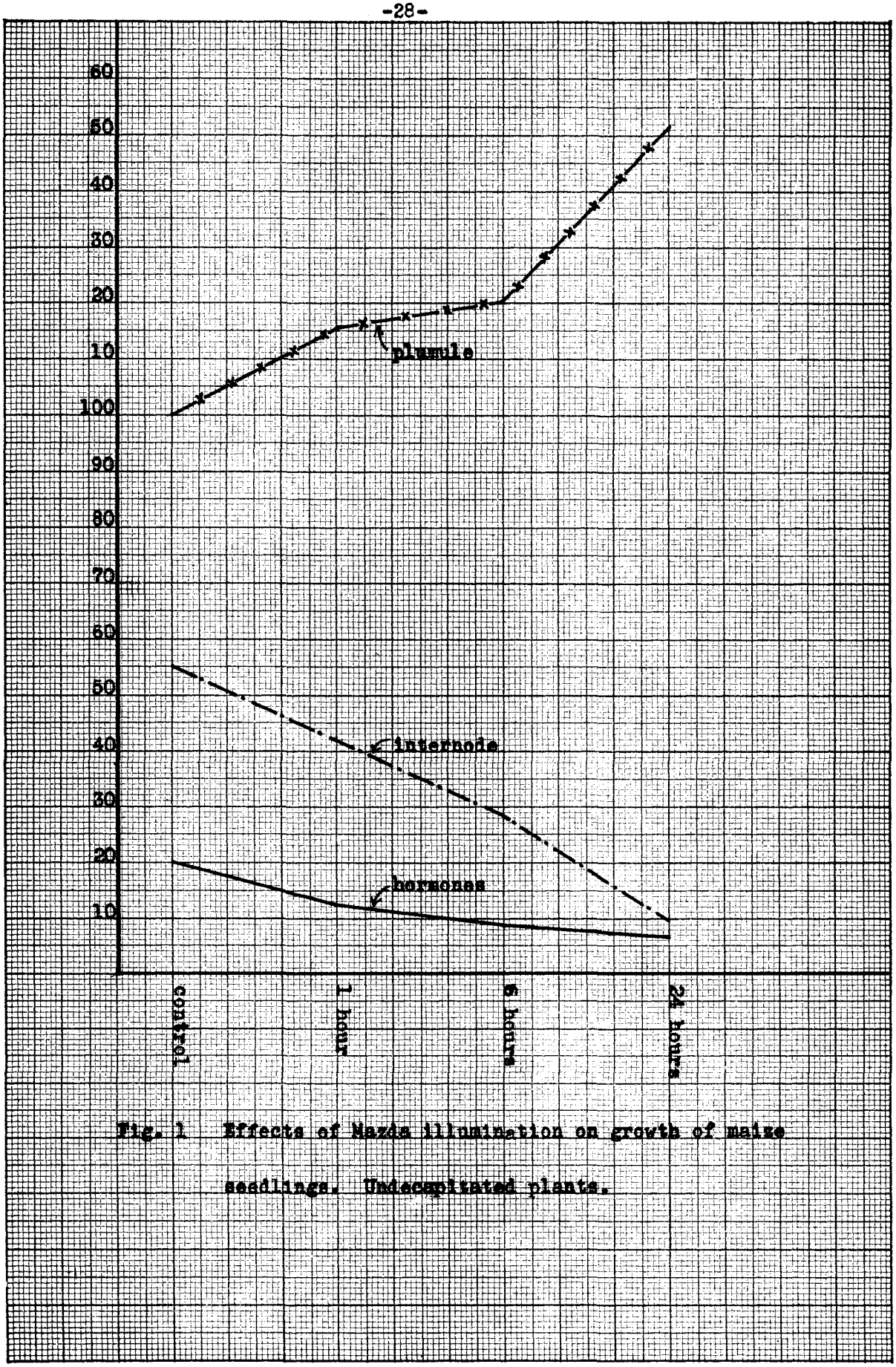


Fig. 1 Effects of Mazda illumination on growth of maize seedlings. Undecapitated plants.

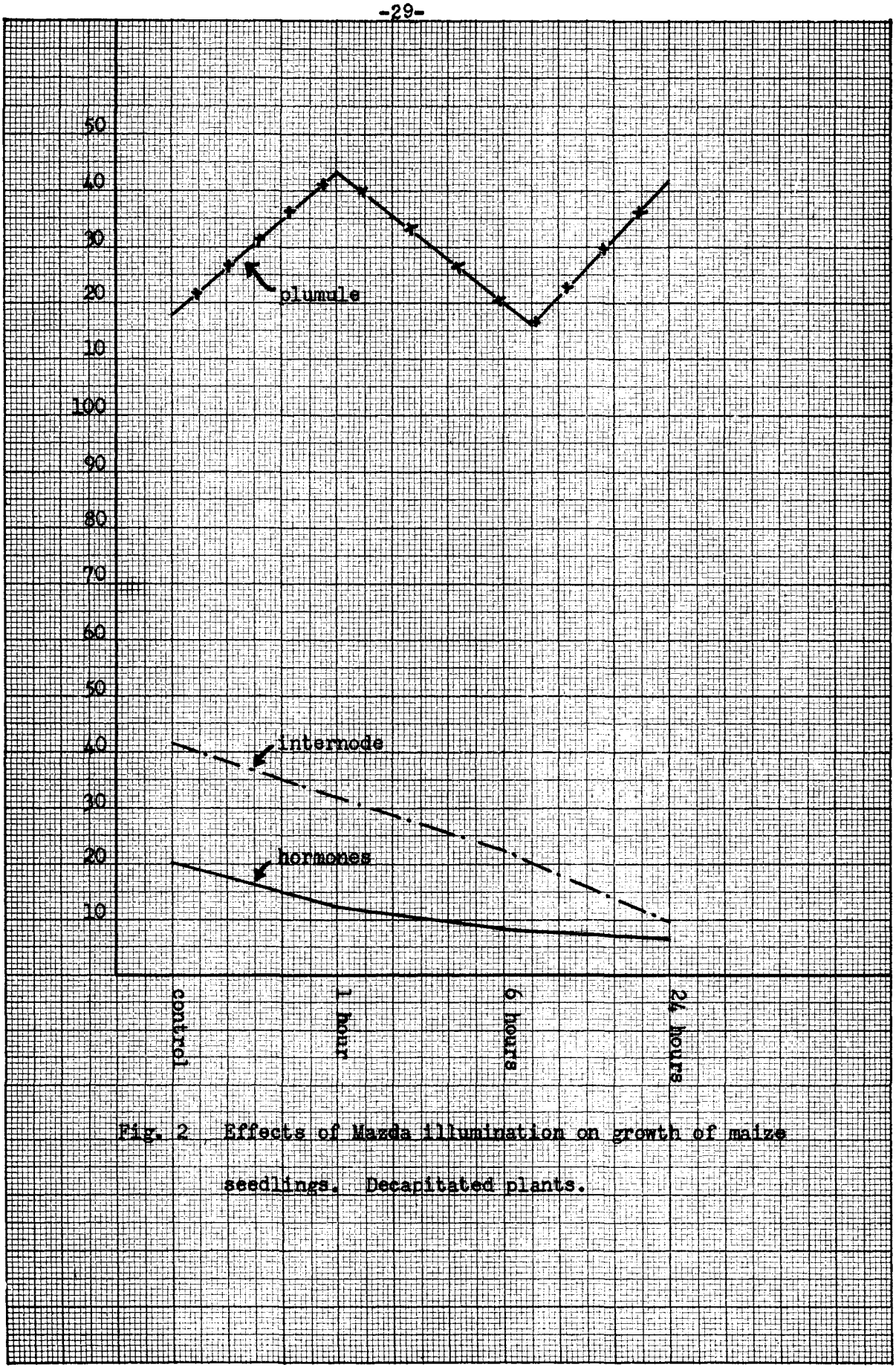


Fig. 2 Effects of Mazda illumination on growth of maize seedlings. Decapitated plants.

were compared with those illuminated twenty-four hours. There was possibly a trend toward increase in plumule growth in decapitated plants also, though the differences were not statistically significant. Interference by decapitation could have had effect.

In no case were there significant differences in the growth of the axis. Controls in the dark showed more internode and less plumule growth. Plants illuminated twenty-four hours made almost no internode growth, but plumule growth slightly more than made up the loss so that the total length of the axis was not decreased.

Hormone measurements showed a progressive decrease with illumination. Analysis by pairs showed the decrease in amount of hormone to be significant when plants illuminated six hours and twenty-four hours were compared with controls. Although the figures representing total degrees curvature showed a decrease in hormone content for one hour illumination and between one and twenty-four hours illumination, the results were variable and calculations did not show these differences to be significant.

The results of these experiments show that light striking the coleoptile of maize caused a shift from internode to plumule growth and reduced hormone activation at the tip. The data are in accord with the work of Inge and Loomis (24), Van Overbeek (52), and Goodwin (22), and lend support to an indirect mechanism for inhibition of growth of the mesocotyl, as opposed to the direct mechanism proposed by Schneider (40).

Experiments with a carbon arc lamp. The corn for these experiments was grown on moist blotters in germination trays which were kept in moist chambers in the darkroom. Except during the experimental period of il-

lumination the seedlings were never exposed to any light but the photo-tropically inactive red light necessary for manipulations.

When the plants averaged 2.5 centimeters in height, uniform seedlings were selected and divided into groups of at least twelve plants, so that a convenient number of coleoptile tips would be available for hormone tests. One group was designated as controls, the others as plants to be illuminated one, three, ten, or twenty minutes. With every experiment an effort was made to perform a complete test, that is to furnish enough corn and Avena coleoptiles to have each experimental period of illumination represented in hormone tests.

Corn to be used as controls was labelled, measured, and left in the dark. Seedlings to be irradiated were measured and then arranged in a semicircle around a carbon arc lamp, fitted with Eveready Supertan carbons No. 389, at a distance of one foot from the arc. This distance gave a measured light intensity of about 50 foot candles on a Weston meter with an energy distribution high in ultra-violet rays. Immediately following a light treatment the coleoptile tips were removed from the illuminated plants, placed on plates of agar, and tested for hormones according to the standard procedure. The decapitated seedlings were then returned to the dark chamber. Half of the control plants were decapitated for hormone tests and half were left intact. Both lots were held for growth measurements.

The experiments contained two imperfections which should be remembered in interpreting the results; hormone tests were started at once after irradiation instead of the two hours afterward which should have given the minimum hormone readings (cf. Table 3), and the effect of decapitation was not separated from that of irradiation.

Growth measurements were made a second time from one to five days after the treatment; most of the second measurements were made three and four days after the first measurement. In Table 2 are summarized the effects of carbon arc illumination on the hormone concentration of all seedlings whose parts were measured both before and after the period of illumination. It, therefore, includes a complete summary of growth and hormone measurements. These results are graphically represented in Figure 3.

Table 2. Summary of Tests Using Carbon Arc Illumination

Length of plant parts is expressed in millimeters. Hormone test measurements are in terms of total average curvature of oat plants.

	Axis	Internode	Plumule	Hormone test
Control				
Intact	60.8	34.8	26.0	14.8
Decapitated	62.1	26.0	36.1	----
One minute	52.1	22.2	29.9	11.7
Three minutes	49.9	19.8	30.1	10.3**
Ten minutes	51.3	19.9*	31.4	10.3**
Twenty minutes	58.1	17.4*	40.7	9.9**

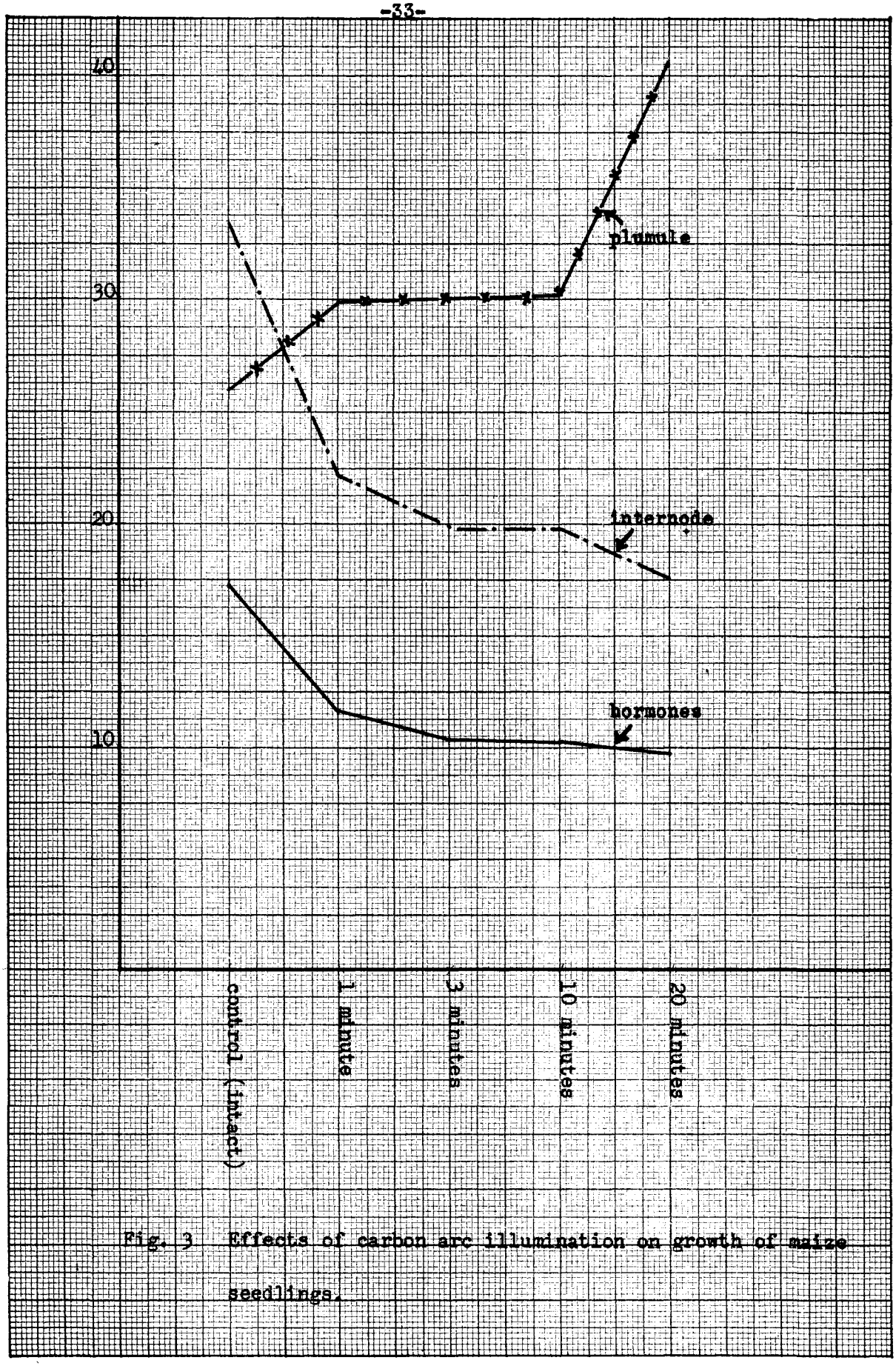


Fig. 3 Effects of carbon arc illumination on growth of maize seedlings.

By analysis of pairs, hormone tests showed a highly significant difference between three, ten, and twenty minutes of carbon arc illumination compared with controls. There was a significant difference in the growth of the internode of plants irradiated three, ten, and twenty minutes, compared with intact controls. The hormone differences are probably minimum because of the short elapsed time allowed for the irradiation effects. They show the rapid reduction in hormone production that can be obtained with irradiation high in ultra-violet rays.

The growth measurements are of less value than those of Table 1, because no irradiated but intact plants were used. Nevertheless the internode showed a progressive decrease in length with increasing amount of illumination. The figures indicated a tendency toward constant increase in length of the plumule. Plumule results were variable and not statistically significant. More tests would probably have established the significance of the trend shown in the data.

Controls showed greatest internode growth and least plumule growth, while plants illuminated twenty minutes showed least internode and greatest plumule growth. Apparently in the shift from internode to plumule growth, then, the internode decreased at the same time the plumule showed a definite increase. With these two processes operating together the length of the axis remained practically the same.

Since the hormone content decreased during the shift from internode to plumule growth, these data seem to furnish further evidence for an indirect mechanism operating in the correlative development of the seedling. Apparently light reduced the hormone activation at the coleoptile

tip in such a way as to induce an important growth shift. These results substantiate the work of Inge and Loomis (24), and are in harmony with the theories of Van Overbeek (52) and Goodwin (22) relative to the effect of light on the tip of the coleoptile.

The Role of the Coleoptile Tip in the Correlative Development of Maize Seedlings

Injury and recovery of the coleoptile tip. As a test of injury and recovery of the coleoptile tip, corn, grown on germination trays as in the previous experiments, was illuminated with the carbon arc lamp after it had reached an average height of three centimeters. Results obtained are shown in Table 3.

Table 3. Hormone Content of Corn Coleoptile Tips at Varying Periods after a Ten Minute Irradiation with a Carbon Arc Lamp

Sampled	Total average curvature
Immediately after	17.6
Two hours after	9.9
Four hours after	9.9
Eight hours after	13.2
Twenty-four hours after	26.0
Forty-eight hours after	29.6

These results indicate that following a period of illumination there was a definite drop in hormone content. After eight hours there was a partial recovery, however, and by twenty-four hours the total average curvature exceeded that of the test immediately following illumination. A comparison with the data of Table 2 indicates that the values reached after 24-48 hours were probably those which would have been obtained before illumination. These data show a recovery of treated plants comparable to that shown for decapitated plants (24), and indicate that relatively short periods of low hormone concentration are capable of causing a permanent shift from internode to plumule growth.

The interaction of coleoptile and internode as affected by decapitation, auxin, and aging. In order to have "young" and "old" seedlings for each test, two sets of corn were germinated in sterilized sand. In two of the three replicated experiments the "old" corn was six days old, in the other, seven days. In each case the "young" corn was four days old. Both the "young" and "old" plants were treated in nine different ways. These were designated as:

Young uncut	Old uncut
Young cut and replaced	Old cut and replaced
Young cut short and replaced	Old cut short and replaced
Young cut plus agar only	Old cut plus agar only
Young cut short plus agar	Old cut short plus agar
Young plus auxin paste	Old plus auxin paste
Young cut short plus auxin paste	Old cut short plus auxin paste
Young plus old tip	Old plus young tip
Young cut short plus old tip	Old cut short plus young tip

Explanation of the above treatments is as follows:

Uncut - coleoptile intact throughout
Cut - about two millimeters of the tip removed
Cut short - at least one centimeter of the tip removed
Cut and replaced - tip replaced immediately after the cut - sealed back on with melted 1.5 per cent agar
Agar only - application of plain 1.5 per cent agar
Auxin paste - application of 1:400 indoleacetic acid in lanolin
Old tips - tips from the old set of plants cemented onto the decapitated young plants with agar
Young tips - tips from the young set of plants cemented onto the decapitated older plants

The plumule was removed from those plants cut short; this operation seemed desirable, since rapid growth of drooping plumules made applications to the tip and measurements inconvenient.

At the time of the operations centimeter marks with India ink were placed on the internode of each plant, starting at the coleoptilar node. In some cases, for closer examination, millimeter marks were made. Growth measurements were recorded after twenty-four and forty-eight hours. All operations were performed in the photographic darkroom and no light reached the corn from the time it was planted until final measurements, except the phototropically inactive red light used during the operations. Manipulations were performed as quickly as possible so that seedlings treated in the various ways would be, in so far as possible, at the same stage of development.

Since growth measurements were from one centimeter marks, one centimeter was subtracted from the final measurements of each segment, so that results represent increase in growth above the original. Data for this experiment are recorded, in terms of the total growth of the internode above the original, in Table 4, and graphically represented in Figure 4.

Table 4. The Effect of Various Treatments of the Coleoptile Tip upon the Growth of the Internode

Treatments	Test 1		Test 2		Test 3		Average
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	48 hours
Young plants							
Cut short + old tip	0.6	0.7	0.5	0.7	1.6	1.6	1.0
Cut short and replaced	0.8	1.0	0.6	0.6	2.0	2.4	1.3
Cut short + agar	0.9	1.4	0.5	0.5	1.9	2.1	1.5
Cut + old tip	1.1	1.5	1.3	1.9	2.7	3.3	2.2
Cut + agar	1.2	2.2	1.2	1.4	2.2	3.1	2.2
Cut + auxin	1.3	1.7	1.1	1.2	4.1	5.4	2.8
Cut and replaced	1.3	2.0	1.0	1.0	1.6	1.8	1.6
Cut short + auxin	1.4	2.0	0.5	0.5	3.8	4.5	2.3
Uncut	2.5	3.7	1.1	1.2	3.8	4.5	3.1
Old plants							
Cut short + young	0.8	1.1	0.0	0.0	0.0	0.0	0.0*
Cut short and replaced	1.1	1.5	0.0	0.0	0.0	0.0	0.0
Cut short + agar	0.4	0.9	0.0	0.0	0.0	0.0	0.0
Cut + young tip	0.6	0.9	0.0	0.0	0.0	0.0	0.0
Cut + agar	0.9	1.6	0.0	0.0	0.0	0.0	0.0
Cut + auxin	1.5	2.9	0.0	0.0	0.0	0.0	0.0
Cut and replaced	0.9	1.7	0.0	0.0	0.0	0.0	0.0
Cut short + auxin	1.2	2.0	0.0	0.0	0.0	0.0	0.0
Uncut	1.9	3.0	0.0	0.0	0.0	0.0	0.0

*Results of test 1 omitted.

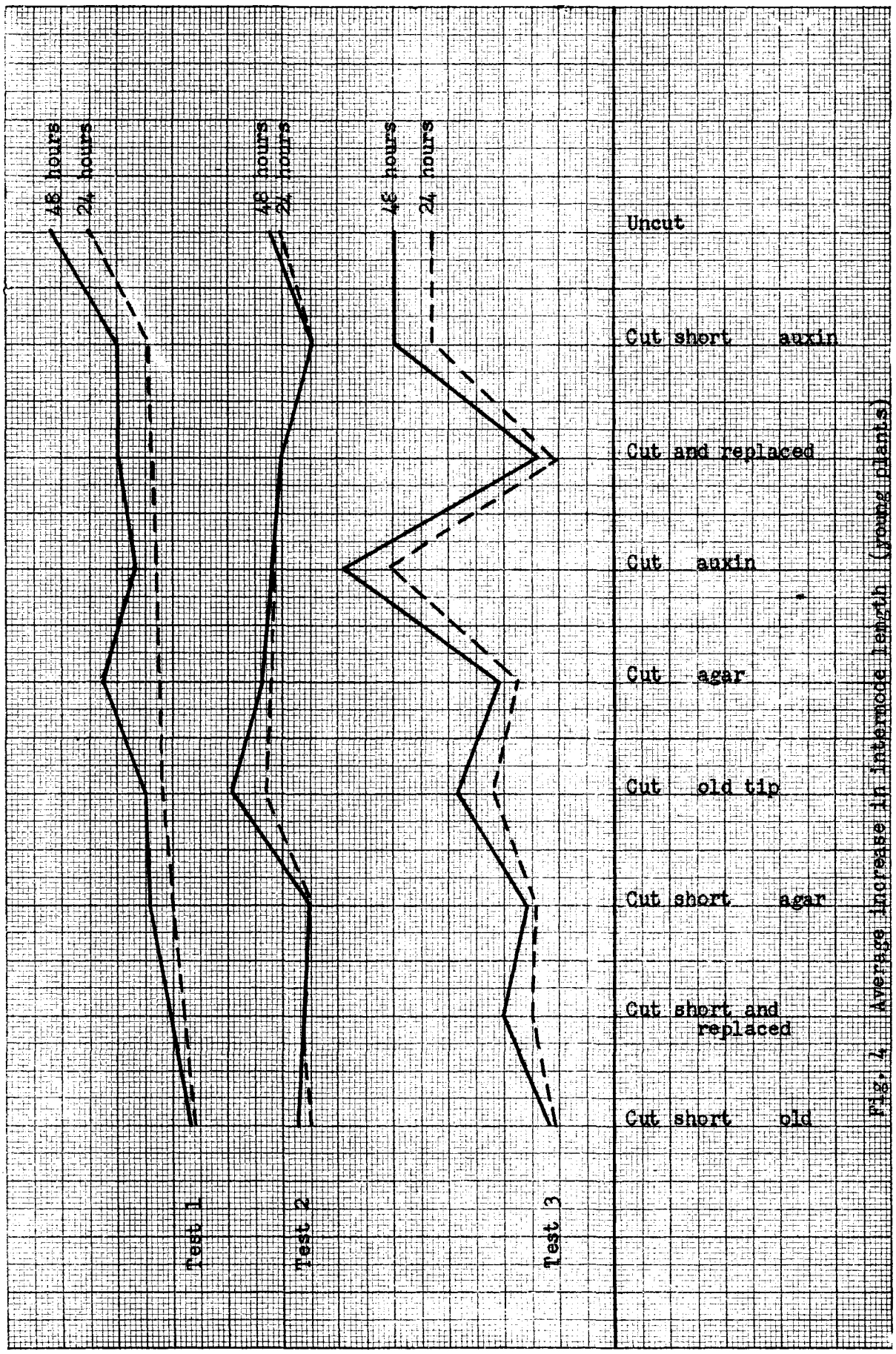


Fig. 4 Average increase in internode length (young plants)

It was noted that in test 1 the "old" seedlings showed growth of the internode above the original one centimeter mark, but this was due to the fact that these plants were not really old, that is, for environmental reasons (lower temperature) they had not aged as much as the six day old plants used in test 2.

An analysis of variance of the data from the young plants is given in Table 5. It was not considered desirable to include the old in the analyses, since the plants which were unquestionably old showed no growth.

Table 5. Analysis of Variance Computed from Data of Table 4.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	53	73.87		
Time	1	1.97	1.97	4.4*
Treatment	8	18.51	2.31	5.2**
Replications	2	37.16	18.58	42.2**
Treatment x time interation	8	0.97	0.12	0.27
Error	34	15.23	0.44	

*Significant
**Highly significant

Differences due to time (24 vs. 48 hours) were significant, while those due to treatment and replications were highly significant. The variability of the results is indicated by the large mean square attributable to replications (repeated experiments).

In general the results of these experiments show that auxin, either from an actively producing coleoptile tip or from a synthetic source, promotes the growth of the first internode. Removing 1 cm. or more of the coleoptile gave the minimum internode growth, an average of 1.0 cm., but the same cutting plus auxin paste gave a growth of 2.3 cm. In general treatments in which auxin paste was used or which favored rapid regeneration of the tip resulted in the greatest growth. There is no evidence that cementing either new or old tips onto cut coleoptiles had any greater effect than the agar alone, which presumably reduced drying of the tip and hastened regeneration.

The node between the coleoptile and internode served as a guide for placing marks to study growth of the internode; the first India ink mark was placed on this node, and all other marks were gauged from there down. Growth in Table 4 was measured between the internode and a single mark 1 cm. below. Figures to show the distribution of growth in the internode over millimeter marks below the internode are presented in Table 6. Centimeter measurements showed growth confined to the first centimeter; and millimeter measurements show greatest growth in the first millimeter of the internode below the coleoptilar node. Continued growth of the tenth millimeter of the internode of young uncut plants is notable, since in most cases growth stopped at the sixth millimeter.

Changes in hormone content with age of the coleoptile. In the experiments so far reported the coleoptile tip has been illuminated or otherwise treated and the accompanying shift from internode to plumule growth has been shown to be associated with at least a temporary decrease in the

Table 6. The Distribution of Internode Growth over Millimeter Intervals Below the Node, Seedlings with Untreated Coleoptiles Compared with Coleoptiles Treated as in the Previous Experiment

Data are Millimeters

mm.	Uncut	Cut and replaced	Cut short and replaced	Cut plus agar	Cut plus old tip	Cut short plus old tip
1	12.1	7.5	2.5	6.0	2.6	2.7
2	6.0	4.8	1.5	3.5	1.8	1.8
3	4.0	2.6	1.1	2.0	---	0.9
4	2.5	1.7	0.7	0.9	1.0	0.4
5	1.5	1.2	0.5	0.4	0.4	0.3
6	1.0	0.9	0.0	0.1	0.3	0.2
7	0.8	0.6	0.0	0.0	0.0	0.0
8	0.7	0.4	0.0	0.0	0.0	0.0
9	0.6	0.1	0.0	0.0	0.0	0.0
10	0.1	0.0	0.0	0.0	0.0	0.0
Total	29.3	19.8	6.3	12.9	6.1	6.3

hormone output of the coleoptile tip. If untreated maize plants are germinated in darkness the internode elongates abnormally, pushing the coleoptilar node far above the soil level, but it stops in 5 to 8 days and the plumule and nodal roots develop as though the plants had been irradiated. This shift is preceded by aging of the coleoptile tip, and it was considered possible that a reduction in hormone production also preceded the shift.

Daily hormone tests were made to study the change in hormone content with aging of the coleoptile. Sufficient pots of corn were planted in sterilized sand to provide material for each day. The tests for hormones were performed according to the general procedure outlined in the section on materials and methods. These tests are summarized in Table 7 and graphically represented in Figures 6 to 9.

These results show that hormone production by coleoptiles decreased with aging. The change could not be described as gradual, however; the hormone remained relatively high and then definitely and sharply dropped to near zero.

In all tests internode growth was very rapid at first, followed by a decrease in rate of growth until cessation. The most rapid internode growth occurred in the early development of the seedling, within the first two or three days as given in these data. This period of rapid growth was several days before the definite drop in hormone. It coincided with the maximum hormone production in tests 1, 2, 4, and 5; and in test 3 it corresponded with a hormone measurement which closely approached the maximum. There is not, however, sufficient justification for stating that there was a marked change in hormone at the time internode growth leveled off in

Table 7. Summary of Tests Showing Changes in Growth and Hormone Content with Age of the Coleoptiles

Figures represent average measurements: in millimeters for plant parts, in total degrees curvature for hormones.

Day	Axis	Internode	Coleoptile	Plumule (above the coleoptile)	Hormone
Test 1					
1	46.0	30.0	16.0	0.0	23.6
2	93.7	66.4	27.3	0.0	25.9
3	159.9	111.0	47.1	1.8	28.2
4	253.5	136.7	65.2	52.6	17.0
Test 2					
1	66.7	48.3	18.4	0.0	18.6
2	207.4	127.3	56.5	23.6	23.5
3	238.0	140.7	62.7	34.6	23.5
4	302.0	148.5	63.2	114.7	3.8
5	322.1	148.5	70.5		1.7
Test 3					
1	20.0	13.0	7.0	0.0	18.5
2	118.4	86.3	32.1	0.0	24.6
3	172.2	129.4	42.8	0.0	21.1
4	221.4	154.8	58.4	8.2	28.5
5	246.2	154.8	64.4	27.0	20.8
6	293.7	154.8	67.4	71.5	5.8
7	348.9	154.8	67.4	123.7	1.2
8		154.8	67.4		0.5
Test 4					
1	26.1	18.6	7.5	0.0	20.2
2	109.8	81.0	28.8	0.0	31.6
3	175.3	126.3	49.0	0.0	27.9
4	217.1	126.3	67.3	23.0	18.4
5	285.6	126.3	67.3	92.0	0.88
Test 5					
1	16.9	10.2	6.7	0.0	18.6
2	79.4	54.8	24.6	0.0	27.2
3	156.5	108.6	42.3	5.6	18.7
4	208.9	134.9	56.9	17.1	22.0
5	256.0	134.9	62.3	58.8	11.0
6	288.0	134.9	62.3	90.8	1.9
7	333.8	134.9	62.3	136.6	0.42
8	372.1	134.9	62.3	174.9	0.25

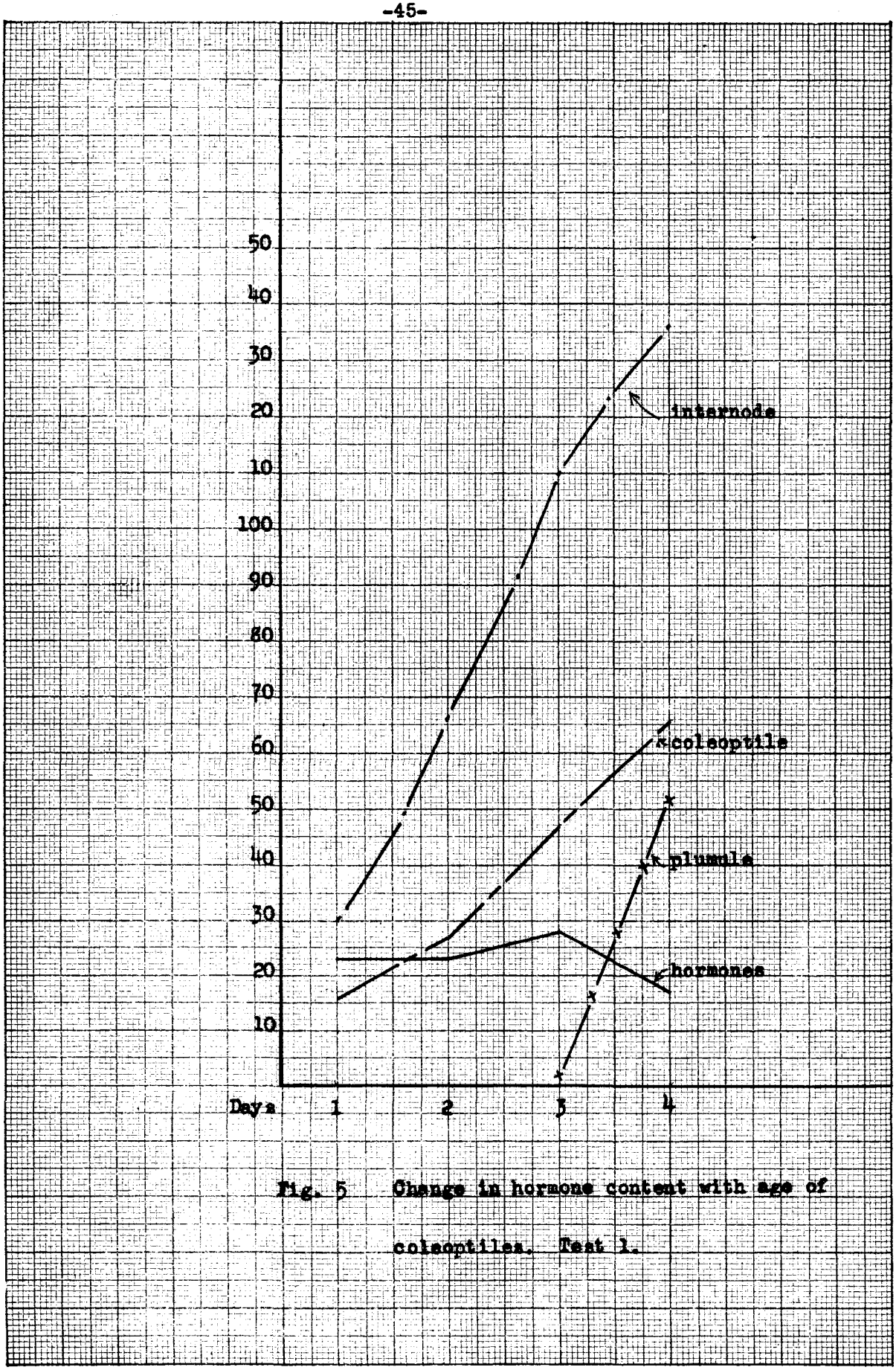


Fig. 5 Change in hormone content with age of coleoptiles. Test 1.

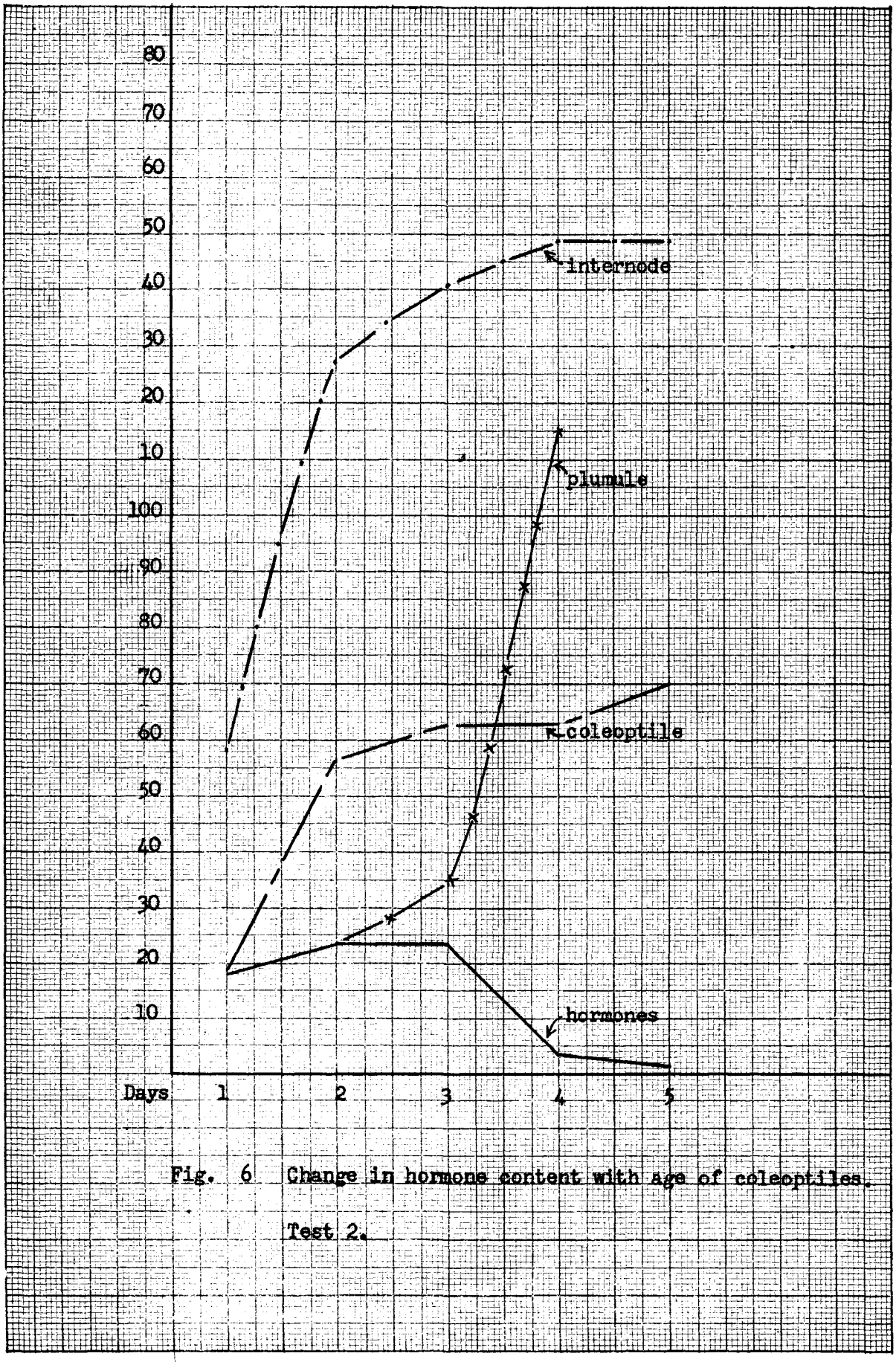


Fig. 6 Change in hormone content with age of coleoptiles. Test 2.

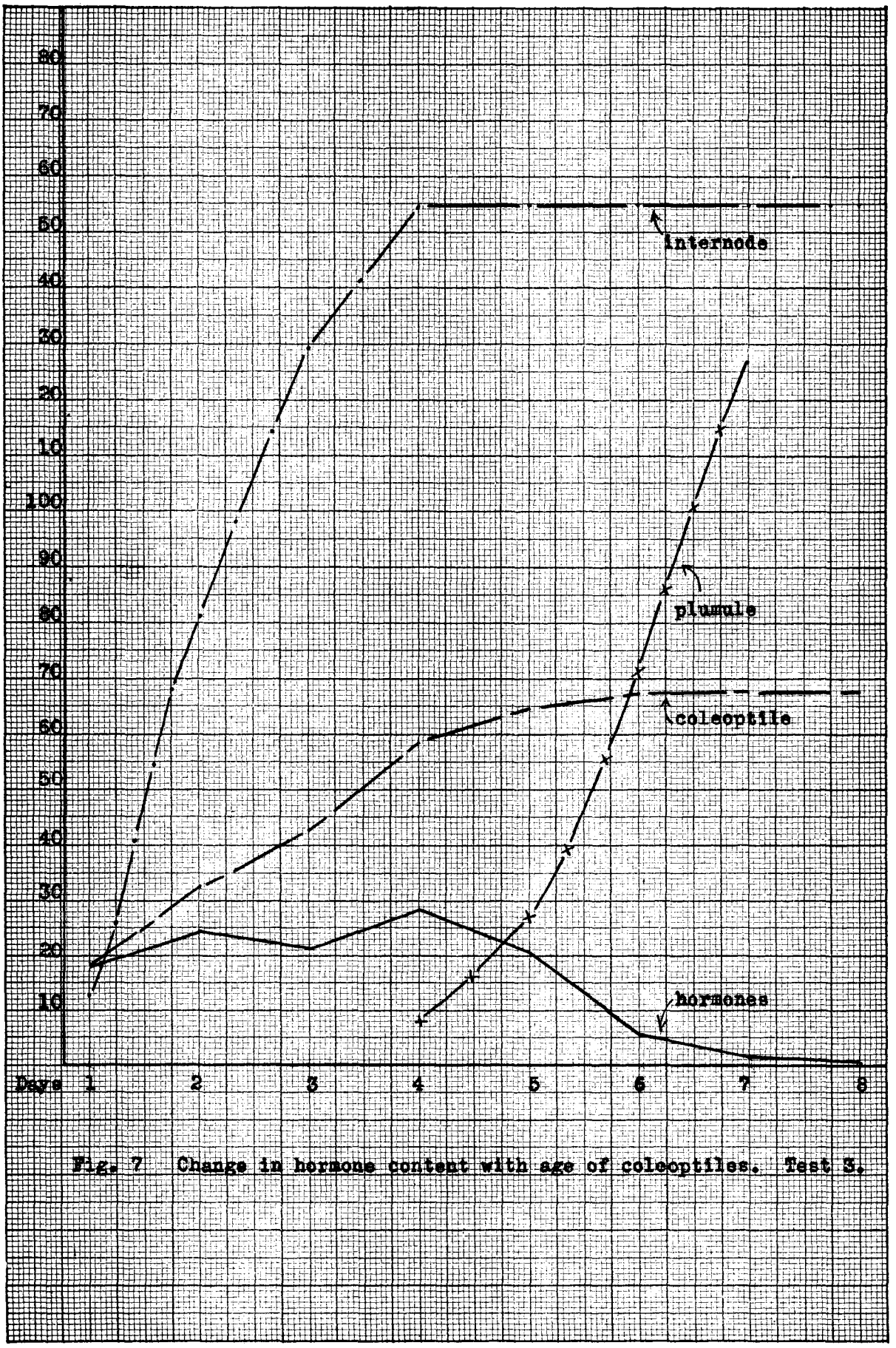
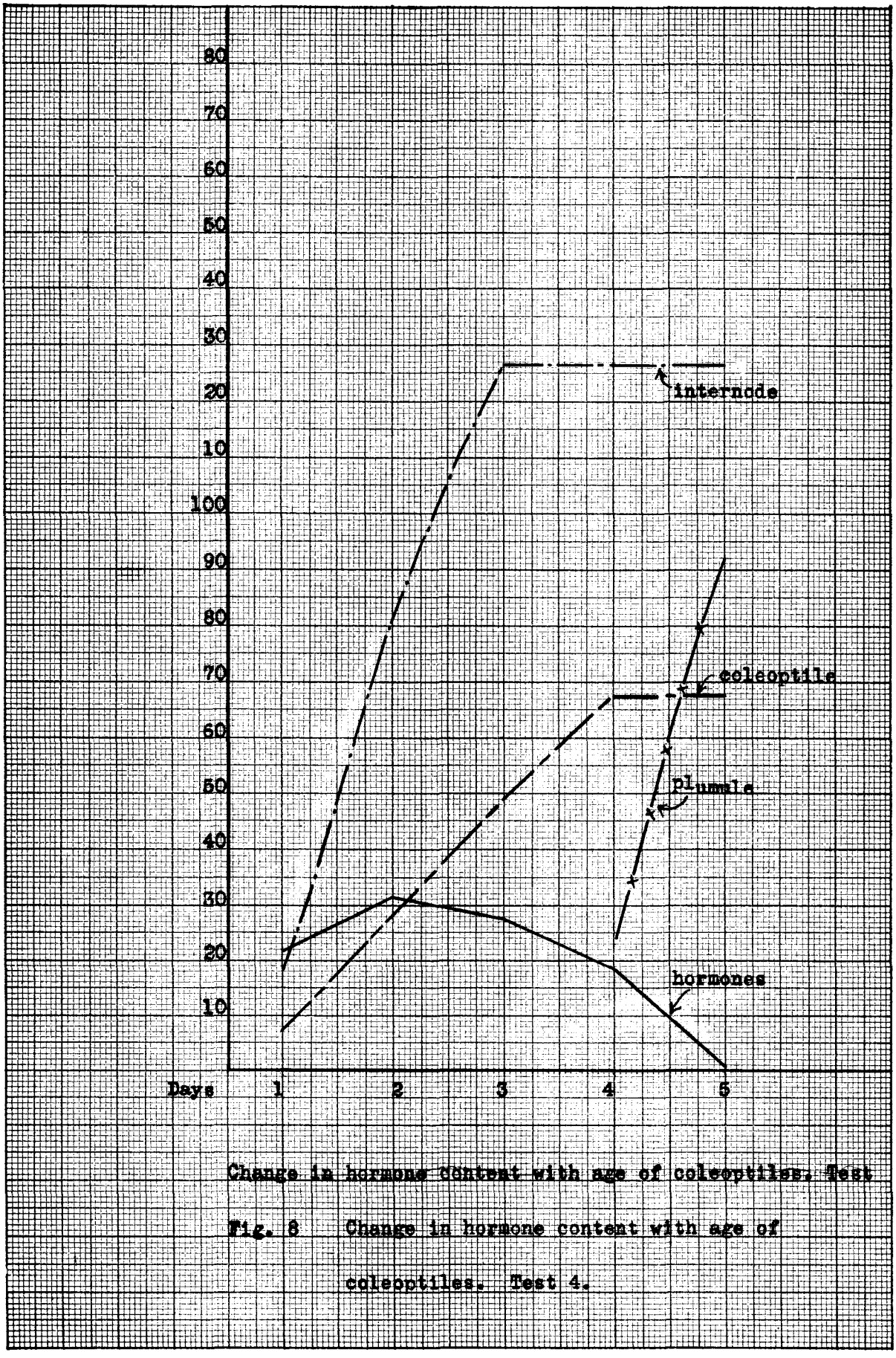


Fig. 7 Change in hormone content with age of coleoptiles. Test 3.



Change in hormone content with age of coleoptiles. Test

Fig. 8 Change in hormone content with age of coleoptiles. Test 4.

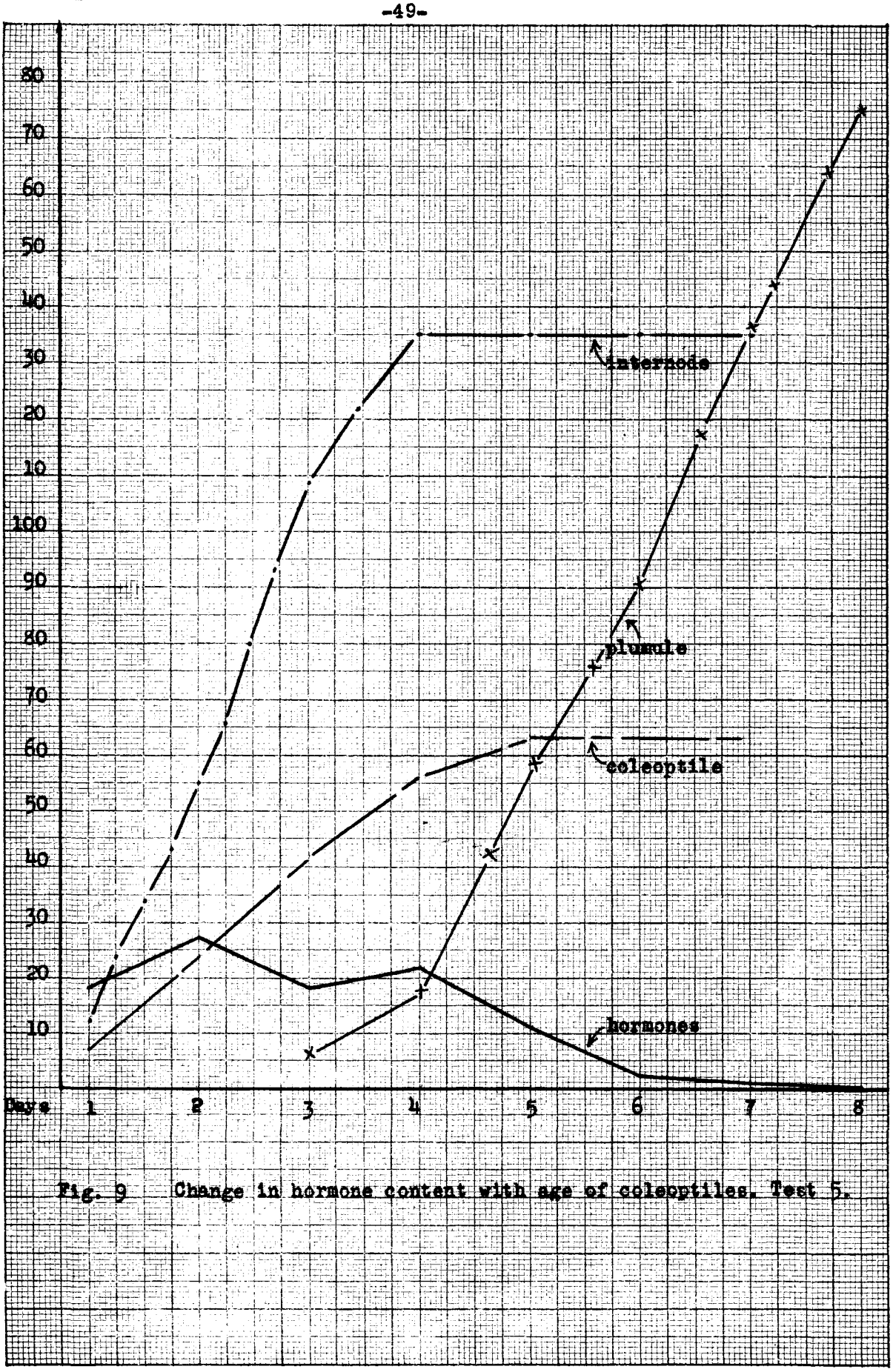


Fig. 9 Change in hormone content with age of coleoptiles. Test 5.

intact, untreated plants grown in the dark. In all the tests except test 2, the hormone was still high at the time internode growth ceased; there were indications of a decline in hormone, which typically dropped to a low figure immediately afterward, but the noticeable drop had not yet occurred at the time internode growth stopped.

Greatest increase in growth of the coleoptile occurred at the time the internode showed greatest growth increment. Further growth of the coleoptile was less rapid than that of the internode, and ceased later. Therefore, by the time growth of the coleoptile had obviously decreased, hormone test measurements had diminished. There was, as in the case of the internode, no clear-cut relationship between the time coleoptile growth leveled off and the definite drop in hormone.

In every test the plumule emerged before the definite drop in hormone. This emergence occurred at the time of maximum hormone in tests 1, 2, 3, and 5; and in test 4, the amount of hormone closely approached the maximum. At the time of the abrupt hormone drop the plumule was growing rapidly.

To support the hypothesis that reduction of hormone activity in the tip is responsible for the shift from internode to plumule growth, one should establish a definite relationship between the marked change in hormone content, the time the internode levels off, and the time of emergence of the plumule. These experiments in the dark do not give clear-cut evidence of such a relationship.

Then, some other factor than hormone appears to operate in the shift in growth. The function of auxin is probably more direct in the early development of the seedling, as evidenced by the fact that the most rapid

internode and coleoptile growth occurred at the time of greatest hormone content, but apparently other factors associated with the age of the coleoptiles operate in later changes.

Growth Correlation and Hormone Content

Further experiments were performed to study the correlative development of internode, coleoptile, and plumule, and hormone tests. Particular attention was paid to the point at which mesocotyl growth stopped and whether the growth of the mesocotyl was reversible once it had stopped.

Twenty-one pots of corn were planted in sterilized sand in the darkroom with twelve plants in each pot. Operations were performed on separate lots of plants on each of four days after germination. Every experiment involved the use of three pots of plants, so that each of three groups would be represented. Seedlings of group 1 were untreated controls; 1:400 indoleacetic acid paste was applied to the intact tip of plants in group 2. In group 3, 2 to 3 mm. of the tip was removed and auxin paste was applied to the cut tip. The tips removed were used in hormone tests, which were made according to the procedure outlined in materials and methods. Each day the auxin paste was freshly applied to coleoptiles on which it was used.

All operations were performed in the phototropically inactive red light of the darkroom, and throughout the experiment growth took place in the dark.

The treatments were repeated until no further growth occurred in the internode and coleoptile. Careful notes were made on the condition and length of the total internode, coleoptile, and plumule at twenty-four

hour intervals. Growth measurements of the internode were facilitated by centimeter marks with India ink, gauged from the coleoptilar node.

The experiment was repeated four times and results are presented separately for the four series of tests; the three groups mentioned above are in each series. Complete data are given in Tables 8 to 11. The data of the second series of experiments are in addition shown graphically in Figures 10 to 13.

Hormones were studied only in the controls, since these were assumed to represent normal plants. Plants with synthetic auxin were used for comparison of growth responses, particularly to determine whether or not internode growth could be reinitiated after having stopped normally in the course of seedling development.

All tests showed a drop in hormone as the coleoptiles aged. The drop was more definite and abrupt in tests 1, 2, and 4, however, than in test 3.

Noticeable in all tests was a second rise in hormone. When this occurred the internode and coleoptile had both leveled off; the plumule was growing at various rates.

The most rapid internode growth occurred early in the development of the seedling, the second day of these data, and then tapered off. Except in test 4 this growth period coincided with the period of maximum hormone increase. In all tests, however, the most rapid internode growth was four to seven days before the drop in hormone. Leveling off of the internode and coleoptile preceded the abrupt drop in hormone.

Table 8. Growth Correlation in Maize Seedlings -- Series I

All Data are Lengths in mm.

	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone
Day	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	test
				Treatments applied the first day after germination						
1	48.7	29.1	----	50.6	31.4	----	49.6	29.1	----	23.6
2	75.3	41.8	8.2	79.0	56.4	----	80.1	61.5	----	28.6
3	78.0	68.7	48.5	83.1	73.5	----	83.3	83.0	6.0	30.5
4	79.7	72.6	103.1	83.1	80.5	26.7	85.5	86.4	27.1	12.8
5	79.7	72.8	156.4	83.1	80.5	32.4	85.5	86.4	48.7	21.9
6	79.8	74.0	222.1	83.1	80.5	88.1	85.6	86.4	90.7	13.2
7										9.4
				Treatments applied the second day after germination						
2	80.3	50.1	5.9	89.2	53.0	1.0	80.4	56.2	1.7	28.6
3	80.3	66.6	33.8	93.8	78.2	9.0	81.3	76.1	26.2	30.5
4	86.6	70.5	86.0	93.8	86.0	42.8	81.3	86.6	45.6	12.6
5	88.0	70.5	117.0	93.9	86.0	105.0	81.3	86.6	61.6	21.9
6	88.0	70.5	154.1	93.9	86.0	105.0	81.3	86.6	135.3	13.2
7										9.4
				Treatments applied the third day after germination						
3	93.7	64.1	42.1	90.4	63.2	26.6	87.4	63.7	27.0	30.5
4	93.7	67.9	83.6	90.4	75.1	52.2	87.6	71.0	74.2	12.6
5	93.7	68.2	94.6	90.4	75.1	92.0	89.2	71.0	110.1	21.9
6	93.7	68.5	210.9	90.4	75.1	179.6	89.2	71.0	189.5	13.2
7										9.4
				Treatments applied the fourth day after germination						
4	80.9	59.5	64.8	91.8	65.5	78.4	95.7	70.3	76.9	12.6
5	80.9	62.7	132.9	91.8	65.5	135.1	95.7	70.3	118.8	21.9
6	80.9	62.7	173.6	91.8	65.5	187.4	95.8	70.3	201.0	13.2
7										9.4

Table 9. Growth Correlation in Maize Seedling -- Series II

All Data are Lengths in mm.

Day	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone test
	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	
	Treatments applied the first day after germination									
1	21.7	14.1	----	22.0	14.0	----	20.0	14.6	----	28.0
2	44.4	33.0	----	55.5	37.2	----	55.1	39.0	----	28.8
3	62.8	45.7	12.9	67.0	60.0	----	62.6	63.9	----	20.4
4	65.4	57.7	23.9	69.5	81.0	----	63.4	83.8	----	19.3
5	67.0	60.0	65.8	69.5	88.5	1.2	63.8	91.6	12.7	23.7
6	68.8	61.4	106.2	69.5	91.3	36.9	64.4	----	24.1	5.1
7	68.9	61.7	122.9	69.5	92.5	45.3				15.7
	Treatments applied the second day after germination									
2	41.0	26.5	----	44.7	27.5	----	39.2	26.4	----	28.8
3	71.5	49.3	7.2	73.3	55.2	----	65.8	61.4	----	20.4
4	74.8	51.2	28.3	74.4	70.9	----	67.2	84.0	----	19.3
5	75.3	62.6	71.1	75.9	77.4	11.2	67.9	96.1	2.0	23.7
6	75.9	63.0	109.0	76.4	81.8	33.3	70.6	99.9	16.6	5.1
7	76.0	63.0	149.1	78.4	83.7	44.8	70.6	100.4	23.0	15.7
	Treatments applied the third day after germination									
3	83.5	46.3	2.2	77.2	48.9	5.9	77.3	51.2	2.2	20.4
4	86.3	56.4	37.3	83.2	77.2	16.8	82.7	67.2	16.0	19.3
5	87.7	58.9	75.1	83.8	82.0	58.6	82.8	69.4	44.1	23.7
6	88.5	59.5	102.6	85.5	83.0	89.0	83.3	72.0	79.0	5.1
7	88.5	60.1	126.2	85.9	85.2	113.6	83.3	74.3	91.0	15.7
8	88.5	60.1	167.5	85.9	85.2	153.8	83.3	74.3	124.5	
	Treatments applied the fourth day after germination									
4	85.0	62.0	25.7	86.3	55.9	6.8	86.8	59.0	26.2	19.3
5	85.5	65.5	86.9	87.4	63.1	63.0	87.3	64.0	75.3	23.7
6	86.1	67.2	130.0	87.5	67.1	89.0	87.5	69.1	108.6	5.1
7	86.4	67.2	172.5	87.5	68.6	117.9	87.5	70.0	127.5	15.7
8	86.4	67.2	246.0	87.5	68.6	156.7	87.5	70.0	172.3	

Table 9 (Continued). Growth Correlation in Maize Seedling -- Series II

All Data are Lengths in mm.

Day	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone test
	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	
	Treatments applied the fifth day after germination									
5	93.3	60.8	61.1	80.4	50.8	73.8	90.8	50.8	66.6	23.7
6	94.1	62.0	104.1	82.9	58.0	94.1	91.5	59.7	95.7	5.1
7	94.3	62.0	113.1	82.9	58.0	113.1				15.7

Table 10. Growth Correlation in Maize Seedling -- Series III

Data are Lengths in mm.

Day	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone test
	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	
				Treatments applied the first day after germination						
1	19.0	15.0	----	14.6	14.8	----	17.2	13.1	----	17.6
2	57.2	37.5	0.1	59.1	42.0	----	52.2	----	----	30.2
3	75.2	61.2	19.9	77.2	74.5	----	70.9	64.7	----	25.8
4	76.4	66.0	80.0	80.1	92.2	3.3	73.0	77.6	----	23.9
5	76.6	66.4	98.5	81.1	95.3	20.3	73.9	83.0	6.0	23.6
6	76.6	66.6	122.7	81.1	95.7	28.7	73.9	94.9	13.2	25.6
7	76.6	66.6	153.5	81.1	95.7	37.5	73.9	94.9	21.8	18.4
8	----	----	185.0	81.1	95.7	51.9	----	----	39.4	15.9
9	----	----	207.4			72.4	----	----	64.0	10.6
				Treatments applied the second day after germination						
2	41.3	28.7	----	43.2	27.0	----	42.7	28.0	----	30.2
3	73.3	53.5	10.0	78.2	60.9	----	73.8	62.6	----	25.8
4	77.8	62.4	45.5	81.7	77.8	0.1	75.4	89.0	1.0	23.9
5	78.1	66.0	73.4	81.7	77.8	15.5	78.1	95.0	9.4	23.6
6	78.1	67.3	99.4	81.7	82.3	42.8	78.1	96.1	40.2	25.6
7	78.1	67.3	129.5	81.7	82.3	51.2	78.1	96.7	65.6	18.4
8	----	----	154.6	----	----	60.8	----	----	69.4	15.9
9	----	----	----	----	----	----	----	----	----	10.6
				Treatments applied the third day after germination						
3	88.0	42.4	23.3	81.2	37.3	27.8	89.0	26.2	4.3	25.8
4	90.6	61.4	68.8	84.2	60.8	42.9	92.6	75.6	17.3	23.9
5	90.6	62.4	95.6	84.2	69.0	71.7	92.6	81.6	43.8	23.6
6	90.6	62.4	129.8	84.2	70.0	80.7	92.6	81.6	65.0	25.6
7	90.6	62.4	151.6	84.2	70.0	124.0	92.6	81.6	82.8	18.4
8	----	----	175.8	----	----	142.9	----	----	108.2	15.9
9	----	----	222.0	----	----	190.7	----	----	190.1	10.6

Table 10 (Continued). Growth Correlation in Maize Seedling -- Series III

Data are Lengths in mm.

Day	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone test
	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	
Treatments applied the fourth day after germination										
4	81.2	69.3	57.5	97.0	75.3	44.9	92.2	68.6	32.7	23.9
5	81.3	74.4	99.1	97.2	75.7	----	92.2	75.1	90.5	23.6
6	81.3	74.4	138.4	97.3	75.7	104.0	92.2	75.6	129.1	25.6
7	81.3	74.4	169.5	97.3	75.7	142.6	92.9	75.6	150.7	18.4
8	----	----	199.5	97.3	75.7	166.3	----	----	171.5	15.9
9	----	----	----	----	----	187.3	----	----	----	10.6
Treatments applied the fifth day after germination										
5	86.0	66.8	80.2	99.0	65.1	82.0	80.9	73.4	87.3	23.6
6	86.0	66.8	122.8	99.0	65.3	119.4	80.9	77.0	141.5	25.6
7	86.0	66.8	153.0	99.0	65.3	138.0	80.9	77.0	164.0	18.4
8	----	----	177.0	----	----	157.3	----	----	182.0	15.9
9	----	----	197.3	----	----	177.5	----	----	193.1	10.6
Treatments applied the sixth day after germination										
6	105.6	69.5	103.8	100.5	66.6	117.1	82.1	65.5	106.1	25.6
7	105.6	70.3	126.9	100.5	----	163.7	82.1	67.3	145.9	18.4
8	----	----	151.2	----	----	181.9	----	----	180.5	15.9
9	----	----	165.4	----	----	205.3	----	----	189.7	10.6

Table 11. Growth Correlation in Maize Seedling — Series IV

Data are Lengths in mm.

Day	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone test
	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	
	Treatments applied the first day after germination									
1	37.2	21.4	----	42.1	21.5	----	39.9	21.7	----	25.8
2	67.2	50.0	5.0	76.7	46.1	----	74.5	49.4	----	20.0
3	77.2	61.0	13.1	88.7	60.0	----	83.1	61.2	0.5	14.2
4	79.2	66.5	43.5	91.1	76.2	2.1	83.2	84.5	3.8	14.8
5	79.7	67.3	86.9	91.1	82.6	26.7	83.8	85.5	13.5	18.7
6	79.7	67.3	98.6	91.1	85.1	41.5	83.8	89.8	20.3	20.9
7	79.7	67.3	109.3	91.1	85.1	50.0	83.8	89.9	26.3	22.6
8	79.7	67.3	122.2	91.1	85.1	58.7	83.8	89.9	31.7	24.0
9	79.7	67.3	173.3	91.1	85.1	81.1	83.8	89.9	45.2	5.5
	Treatments applied the second day after germination									
2	80.3	44.4	0.3	74.2	44.7	0.9	90.0	49.6	0.2	20.0
3	89.2	55.2	12.0	86.4	64.4	3.9	102.0	61.5	1.5	14.2
4	89.4	61.3	40.0	87.2	79.1	21.0	104.8	78.4	15.0	14.8
5	89.9	61.3	69.0	87.2	82.6	53.1	105.8	83.2	41.5	18.7
6	89.9	61.3	87.7	87.2	82.6	66.0	105.8	83.2	58.5	20.9
7	89.9	61.3	88.7	87.2	82.6	72.8	105.8	83.2	72.0	22.6
8	89.9	61.3	101.0	87.2	82.6	82.0	105.8	83.2	79.8	24.0
9	89.9	61.3	127.8	87.2	82.6	113.1	105.8	83.2	113.5	5.5
	Treatments applied the third day after germination									
3	83.3	48.0	5.4	101.6	52.9	14.5	82.4	54.1	15.0	14.2
4	84.5	57.1	36.7	103.2	64.6	39.0	83.0	63.1	39.6	14.8
5	86.4	59.2	67.1	103.2	70.1	59.3	83.1	66.0	58.6	18.7
6	86.4	59.2	91.0	103.2	73.2	75.8	83.1	66.3	71.1	20.9
7	86.4	59.2	105.5	103.2	73.2	87.2	83.1	66.3	81.5	22.6
8	86.4	59.2	116.1	103.2	73.2	98.0	83.1	66.3	94.4	24.0
9	----	----	----	103.2	73.2	132.0	83.1	66.3	131.5	5.5

Table 11 (Continued). Growth Correlation in Maize Seedling -- Series IV

Data are Lengths in mm.

Day	A - tip uncut			B - auxin on intact tip			C - tip removed + auxin			Hormone test
	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	Internode	Coleoptile	Plumule	
	Treatments applied the fourth day after germination									
4	90.0	57.1	38.1	90.1	64.4	40.9	105.6	65.5	50.1	14.8
5	90.4	60.6	66.0	90.4	67.0	61.3	106.5	67.3	71.8	18.7
6	90.4	60.6	83.4	90.4	67.0	79.5	106.6	67.3	91.1	20.9
7	90.4	60.6	93.7	90.4	67.0	85.6	106.6	67.3	96.3	22.6
8	90.4	60.6	104.0	90.4	67.0	90.2	106.6	67.3	110.5	24.0
9	90.4	60.6	141.4	90.4	67.0	140.5	106.6	67.3	141.3	5.5
	Treatments applied the fifth day after germination									
5	114.8	64.0	67.6	106.1	61.5	62.3	----	----	----	18.7
6	114.9	64.0	71.3	106.1	61.6	76.5	----	----	----	20.9
7	114.9	64.0	94.4	106.1	61.6	89.1	----	----	----	22.6
8	114.9	64.0	103.8	106.1	61.6	99.3	----	----	----	24.0
9	114.9	64.0	129.0	----	----	----	----	----	----	5.5

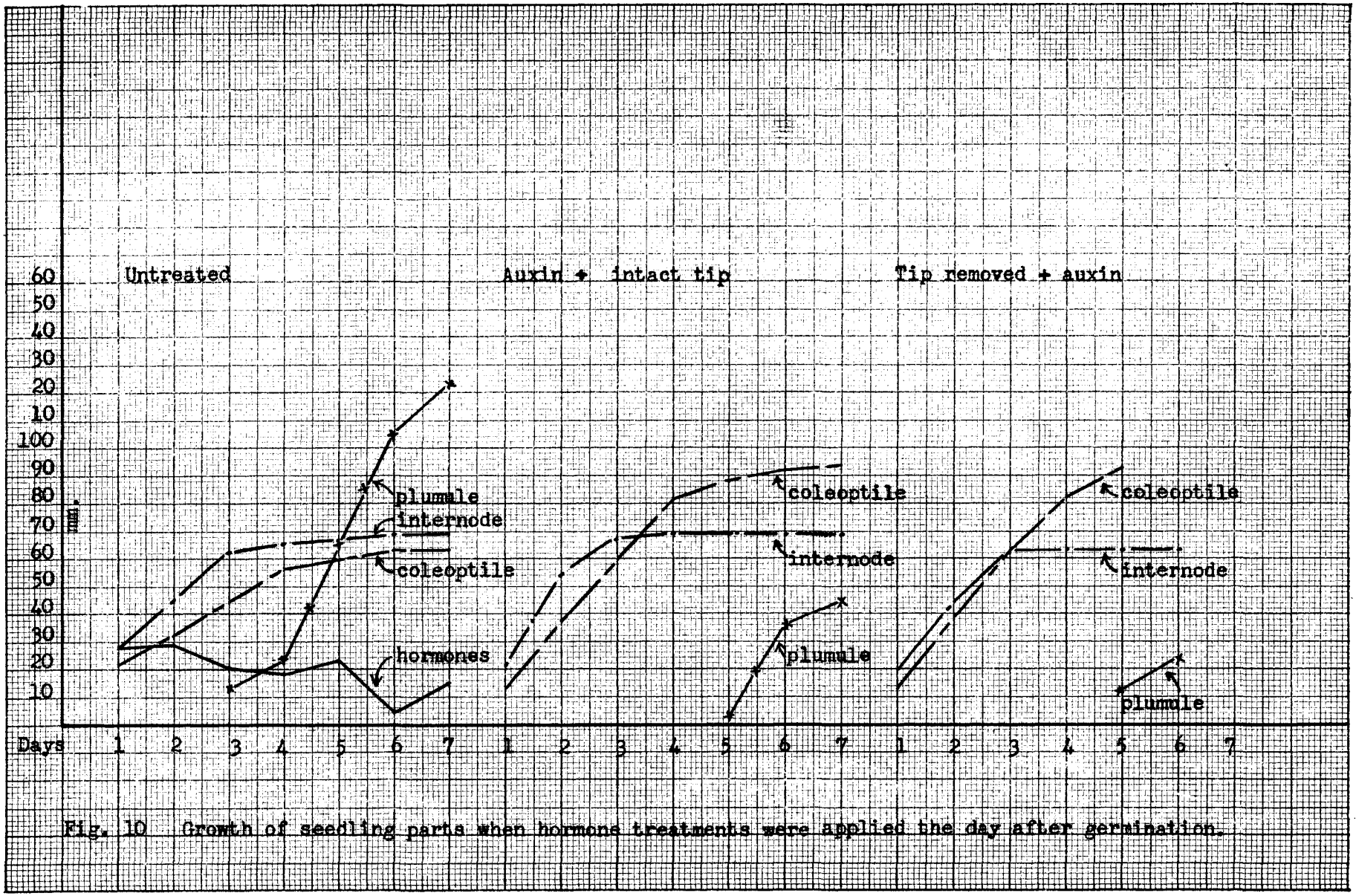


Fig. 10 Growth of seedling parts when hormone treatments were applied the day after germination.

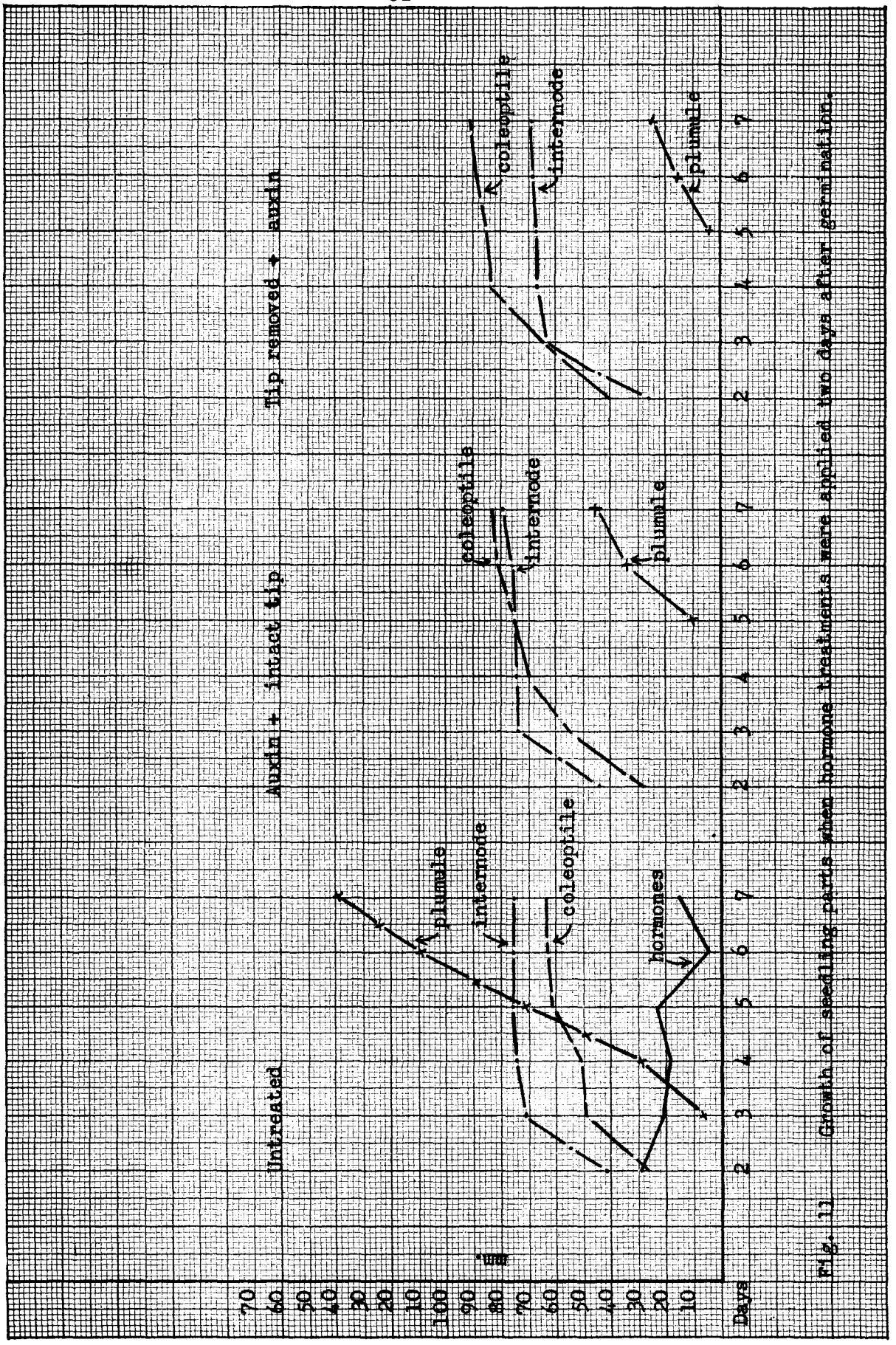


Fig. 11 Growth of seedling parts when hormone treatments were applied two days after germination.

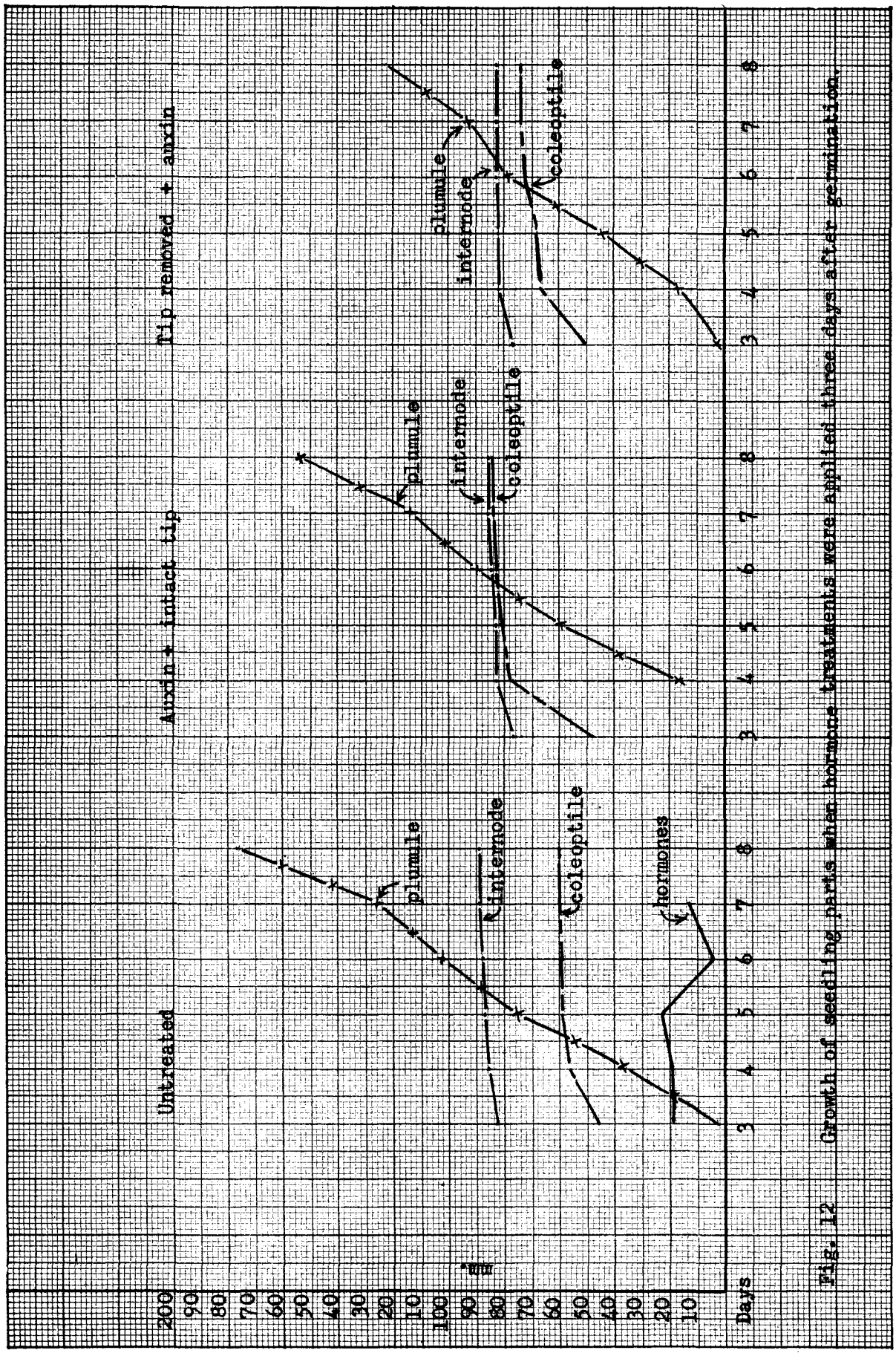


Fig. 12 Growth of seedling parts when hormone treatments were applied three days after germination.

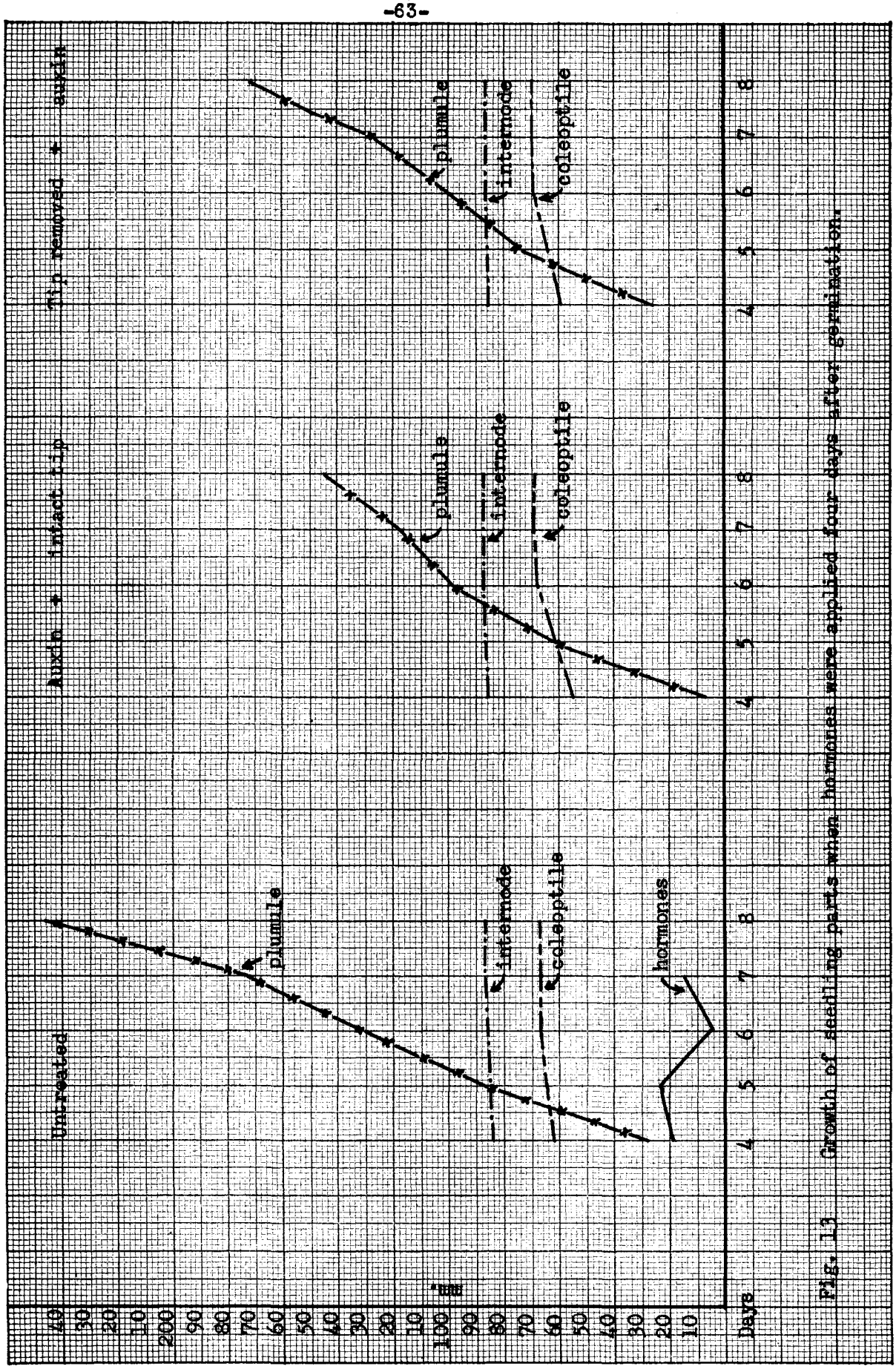


Fig. 13 Growth of seedling parts when hormones were applied four days after germination.

The most rapid growth of the coleoptile took place about the second day. This was at the time hormone was high and internode growth was rapidly increasing. In auxin treated plants the plumule had not yet broken through. Coleoptile growth leveled off about the fourth day -- the same time as the internode growth leveled. The hormone showed some decrease at this time, but not to be compared with the very sudden characteristic drop. In some cases the plumule had not yet emerged.

The rate of growth of the coleoptiles of treated plants was slightly accelerated, compared with the untreated, during the early days of growth. Internode of most auxin treated plants reached a slightly greater total length than the internodes of controls. By contrast the total growth of coleoptiles was decidedly stimulated by auxin treatment.

Both auxin treatments inhibited the plumule, and in both cases the plumule reached an average peak of growth later than it did in the untreated controls. In all groups of the four series the plumule emerged when the hormone was relatively high or, at any rate, before the definite drop in hormone. In the uncut controls the plumule break occurred the second day. Applying auxin to the intact tip and to the cut tip delayed growth of the plumule an average of two days, as compared with controls with tip uncut.

The relation of rate of growth of internode, coleoptile, and plumule with respect to treatment was also studied. Internode and coleoptile reached their peak of growth at the same average time in two days. Although during the early stages of growth the rates of growth of both internode and coleoptile were accelerated by hormone treatments, eventually all leveled off about the same time.

It is notable that once internode growth had stopped it did not start again, even with fresh applications of synthetic auxin. In other words, the change was not reversible.

Notes on nodal roots taken in series 3 and 4 showed that nodal roots appeared the fourth day of testing. This was about the time growth of the internode and coleoptile stopped. The plumule had either not yet appeared, or was not yet growing rapidly.

Considerable curling and distortion took place in the internode and coleoptile of plants treated with hormones during the first three days; this was most marked the first day, and decreased thereafter. Greatest distortion took place in plants in group 3. Every effort was made to obtain accurate measurements in spite of this. The younger the plants when operated upon, the greater the distortion when auxin was applied.

Only plumule measurements above the coleoptile were considered, in view of the fact that often the plumule grew out from the base of the coleoptile, particularly in young, distorted plants.

That it is not auxin reduction alone which controls the shift from internode to plumule growth is indicated by the lack of a clear-cut relationship between the most noticeable drop in hormone, the cessation of growth in the internode, the emergence of the plumule, and the appearance of nodal roots, as well as the irreversibility in the growth of the internode. Superimposed upon the effect of auxin in the correlation of these reactions is the effect of aging which continues to operate in growth changes, including those brought about by hormones.

DISCUSSION

The grass seed, on germinating, develops a shoot. The latter consists of the first internode and of a sheathing structure, the coleoptile, in which the young leaves are enclosed (4). The first internode, which has been variously interpreted, is the portion of the axis between the scutellum and the coleoptile. It elongates by means of division and enlargement of cells just below the level of the divergence of the coleoptile, thus differing from the higher internodes which elongate as a result of activity of intercalary meristems near their base, rather than their top. The coleoptile, a hollow, cylindrical organ, sheaths the growing point and embryonic leaves during the early stages of germination. In the economy of the plant, the elongation of the first internode serves to bring the coleoptilar node, the bases of the foliage leaves, and the apical meristem to an advantageous point within a few millimeters of the surface of the ground. Adventitious roots which anchor the plant in this position then arise at the coleoptilar node (22).

When correlation is defined as the influence exerted by one part of the plant upon another (62), the organs discussed above play an important role in the correlative development of the seedling of grasses. Centers of protoplasm synthesis are also growth controlling centers, and the control mechanism is rather clearly hormonal (59). Primary centers of production of growth regulating substances in plants are root and stem growing points, or other regions where new tissues are being formed with accompanying synthesis of new protoplasm. In seedlings of grasses auxin is produced

in the extreme tip of the coleoptile only; in the mature plant auxin is probably less localized. Excised tips of Zea coleoptiles one millimeter long continued to give off auxin into agar blocks for a period of about twelve hours; those of Avena for about eight hours. The total amount of auxin obtained from one millimeter coleoptile tips by diffusion was always larger than that obtained by extraction, thus proving that auxin is actually produced or activated by the coleoptile tips and not merely given off (58). The growth substance in the tips of grass seedlings is auxin a.

When the coleoptile tip emerges under normal field conditions the first internode development ceases, plumule development is accelerated, and the formation of roots at the coleoptilar node begins. The theory of the mechanism of this growth shift as presented by Inge and Loomis (24) is in accord with that of Van Overbeek (52). The work of these investigators, as summarized by Goodwin (22), shows that internode elongation involves polar elongation of the internode cells; that elongation of cells is contingent upon an adequate supply of auxin; that those treatments, i.e., decapitation, exposure to light, and heat treatment, which inhibit internode elongation, also appear to reduce the auxin supply available for utilization by the internode. The effects of such treatments may be reduced or eliminated by application of a synthetic growth-promoting substance, indoleacetic acid. Here is evidence that inhibition is brought about by a decrease in the supply of auxin available to the internode. It is in support of an indirect mechanism, that light may bring about some photochemical effect in the auxin-producing tip which is subsequently transmitted to the internode below. The research of Schneider (40),

however, suggests that there is also a direct mechanism for the inhibition of the mesocotyl; mesocotyl tissue and coleoptile tissue acting as their own receptors of light stimuli.

The data from the illumination experiments presented in this paper favor the view that an indirect mechanism operates in the shift from internode to plumule growth, without excluding the possibility of a direct mechanism. The results from Mazda illumination show that the internode progressively decreased in length and the plumule progressively increased in length as the hormones decreased. The results in the carbon arc experiments were probably not as clear-cut as those in the Mazda illumination, but they indicated the same trend. For the varying periods of illumination, the time of significant differences between growth measurements and hormone content compared favorably. Considering Mazda illumination, significant differences in plumule measurements and hormone tests were observed when plants illuminated six and twenty-four hours were compared with controls; and in the internode highly significant differences were noted when plants illuminated six and twenty-four hours were compared with controls. In other words, the significant growth differences occurred at the time the hormone test differences were significant.

At no time was growth of the axis significant, either in Mazda or carbon arc illumination. As the shift took place from internode to plumule growth, the internode decreased in length, but the growth of the plumule compensated for the decrease in internode growth. Johnston (25) concluded from illumination experiments that light probably acted more as a redistributing agent of the growth substance than an inactivating agent.

The length of the total axis, in these experiments, might be related to the activity of the growth substance, since it is possible that hormone production was shifted from the coleoptile tip to the plumule in the illuminated plants. At any rate, it is apparent that light striking the coleoptile tip reduced the activity of auxin in the tip. Since at the same time this reduction in activation was taking place the shift from internode to plumule growth occurred, it seems probable that reduction of auxin activity in the coleoptile tip, as a result of the effect of light, was responsible for the growth shift.

Data from decapitated plants were roughly the same as for undecapitated, thus indicating that whether the tips were removed or not, the effect of the illumination persisted after the plants were placed in the darkroom. In the decapitated plants the internode showed less growth, for in addition to having had the light stimulus previous to decapitation, the amount of auxin in the tip of the coleoptile was reduced by decapitation. Van Overbeek (58) found that the growth rate of decapitated Zea coleoptiles dropped during the first two hours after decapitation and increased thereafter; on the other hand, the auxin production of Zea coleoptiles increased with the time after decapitation. Hence, provided there is a precursor still present in the seed and the tip of the coleoptile has not been illuminated to the point of protoplasmic injury, physiological regeneration can take place in the cut tip.

If it is auxin in the coleoptile tip which is responsible for the growth shift one would expect the amount of hormone to decrease, internode growth to decrease, and plumule length to increase, as the individual periods of illumination are compared. This relationship held very well when the

individual periods of illumination were each compared with controls, but not quite so well when they were compared with each other (Table 1). As the individual groups were compared, it could be seen that the greatest hormone decrease occurred at the time of the greatest internode decrease, but not at the time of the greatest plumule increase.

It has been stated that the photochemical change occurring in the coleoptile and transmitted to the internode upon illumination may be either the synthesis of an inhibitor, the destruction of a meristematic stimulator, or the alteration of some transport or synthesis mechanism. The results of these illumination experiments favor the third possibility; that is, a change in the auxin mechanism has a place here. Whereas total growth, and not actual zones of inhibition, was measured, the changes in the auxin-producing tip were substantiated by hormone tests. Evidence indicated that reduction of auxin activity caused by light striking the coleoptile tip was correlated with and probably active in the shift from internode to plumule growth.

Tests for hormones at varying periods following a ten-minute irradiation with a carbon arc lamp (Table 3) indicated that within a two-hour period after the irradiation there was a reduction in hormone sufficient to cause a shift in growth, and comparable to the amount of hormone decrease which occurred immediately after twenty-minute irradiation. After eight hours there was a partial recovery and by twenty-four hours the total average curvature exceeded that of the test immediately following illumination.

The regeneration in growth substance seemed comparable to the type of physiological regeneration which occurs in decapitated plants. When an

Avena coleoptile is decapitated, the center from which growth substance is distributed is cut off; hence the growth rate of the stump is reduced for some time. After a few hours, growth is renewed due to a "physiological regeneration" at the upper end of the coleoptile stump. This phenomenon has been investigated by Dolk (17), Li (30), and others. They showed that the uppermost millimeter of the physiologically regenerated tip is the new center from which growth substance is dispersed.

Apparently, then, either decapitation or illumination for a short period of time, i.e., the ten minutes allowed here, could be sufficient to cause the shift from internode to plumule growth. A long period of reduction in hormone is not necessary for the shift, but a period just sufficient to give the plumule a start.

The experiments on the interaction of coleoptile and internode gave further evidence of how the organs of the seedling might be correlated in growth changes. In general, they showed that auxin, either from an actively producing tip or from a synthetic source, promoted growth of the internode, but only provided growth of this organ had not been halted by a hormone deficiency or by aging. In general, cutting off the coleoptile tip, whether the stump was coated with agar, the original tip or an older tip cemented on the stump with agar, reduced internodal growth below the controls. The addition of indoleacetic acid in lanolin maintained growth at near normal levels but did not prolong it beyond the controls. Removing a larger portion of the tip gave a greater reduction in growth than the usual 2 to 4 mm. decapitation.

No treatment with indoleacetic acid or applied active tips resulted

in the renewal of internodal growth once it had stopped from aging or previous treatment. The results support an hypothesis of a normal aging process which is delayed but not prevented or reversed by a continuous supply of hormone at a high level.

If it is true, as in the illumination experiments, that the shift in growth is caused by reduction of hormone activity in the tip, it should follow that internode growth stops and plumule growth begins when the hormone is reduced, sent, or stated the other way, that a reduction in hormone should precede the normal shift in growth which occurs after 7 or 8 days in intact plants held in darkness. Experiments in the dark did not show this to be the case. An abrupt drop in hormone was characteristic of all tests, but this drop bore no close relationship to the growth measurements. The hormone was high previous to this drop, yet within what was considered high hormone content there was considerable variation. Hormone test measurements were high at the time the plumule broke through the coleoptile, and were still high at the time the internode growth leveled off.

Maximum growth of the internode took place by the second day; as shown by the data this period corresponded to the maximum hormone content. After this time, however, no relationship existed between internode growth and change in hormone. The shift in growth seemed to occur when the seedlings had reached a certain age regardless of, and irrespective of, changes in hormone. The age of the seedling became a dominant factor.

The auxin content at the time of the emergence of the plumule as given in these data is in accord with the work of Van Overbeek on dwarf maize (51). This investigator found that the primary leaf broke through

the coleoptile on the fifth day; the auxin production was still large and had not reached its optimum when this occurred. In these experiments the time of the plumule break coincided with the time of the maximum hormone test measurement in all experiments but the fourth and fifth, during which it occurred after the maximum, though hormone measurement was still high.

Contrary evidence is presented by Söding (43a). When he investigated the distribution of growth hormone at different levels in the light, he found that the tips of old Avena coleoptiles which had been broken through by the foliage leaf contained little or no hormone. Observations made during experiments performed for this paper showed that the hormone content did not reach minimum until some time after the plumule had emerged, when the coleoptile tips were shriveled.

It appears that light can hasten the shift from plumule to internode growth, but in the absence of light the age of the coleoptile is of primary importance.

Bonner (6) described growth as a dual phenomenon consisting of growth in length and growth in weight. Aging is explained in the following manner (61): if cell wall formation and elongation keep in equilibrium, the growth rate will remain constant. If, due to lack of auxin, elongation is checked, cell wall formation will go on, making the cell wall stiffer and less reactive to auxin, or, the cells age. Du Buy (20) was able to show that the growth rate, irrespective of the auxin concentration, decreased when the cells became older. With increasing age the response to auxin decreases for all concentrations of the growth regulators.

SUMMARY AND CONCLUSIONS

When young maize seedlings were irradiated with either Mazda or carbon arc light the hormone output by the coleoptile tip was reduced, internode growth slowed and stopped, and plumule growth was accelerated. The order and magnitude of the changes were consistent with the hypothesis that internode growth of germinating maize is dependent upon hormones activated in the coleoptile tip, and that plumule development is retarded or inhibited by the same hormones. Hormone output by the irradiated coleoptile tips recovered within 24 hours, but the growth shift from internode to plumule, once established, was irreversible.

In experiments with seedlings of different ages, untreated coleoptile tips or added indoleacetic acid prolonged and increased internode growth. But hormone added after growth had shifted from normal aging effects did not reverse the growth. Removing short sections of the coleoptile tip reduced internode growth less than the removal of 1 cm. sections.

Growth of the internode was shown to be due to both cell division and enlargement in the region just below the coleoptilar node. Nearly half of the total growth was made in the first millimeter below the node.

Internode growth stops in from 5 to 8 days in plants held in complete darkness. This growth shift with aging was normally accompanied by a reduced hormone output although the difference was not always clear-cut. Plumule growth and emergence in intact seedlings clearly preceded the normal hormone drop. The data suggest that while the shifts caused by illumination

of young seedlings may be due to a temporary reduction in hormone concentration, other factors are active during aging which eventually bring about the internode to plumule shift in spite of high natural and added hormone concentrations. As before, all attempts to reinitiate internode growth resulted in failure.

The observed reactions can be covered by an aging hypothesis which assumes that internode growth slows with time after germination. High hormone content from the coleoptile tip is considered to retard this aging process but not to stop it, so that internode growth stops after 6 to 8 days at 25° C. in the intact plant in the dark, irrespective of hormone concentration. This growth may be arrested at any earlier date, however, by a temporary reduction in hormone concentration. Once stopped the hormone level that was sufficient to prolong growth is not high enough to restart it. In the same way growth hormones retard plumule and nodal root growth without completely inhibiting them, so that the plumule may emerge from the coleoptile during the period of maximum hormone production. Again, however, a temporary removal of this inhibiting effect will greatly accelerate plumule development.

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